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THE RELATION OF THE VITAMIN B COMPLEX TO RENAL ENLARGEMENT CAUSED BY CYSTINE AND PROTEIN IN THE DIET OF THE RAT*

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VOLUMINOUS work has been done in recent years on the role of protein and protein metabolites in the etiology of kidney disease. The possibility that the kidney might be injured if called upon to excrete the waste products of protein metabolism at a high level of concentration has occurred to numerous investigators. The findings of these investigators have been distinctly controversial in nature.

Newburgh (1) in 1919 was one of the first to attempt to throw light on this question. He found that rabbits' kidneys were injured by a high protein dietary. Squier and Newburgh (2) reported that the kidneys of human subjects were damaged by diets containing a high proportion of protein in the form of beef. Newburgh and Clarkson (3) observed kidney lesions in rabbits which had been subjected to diets containing 36.2 per cent and 26.8 per cent of protein, respectively. The intensity of the lesions appeared to be parallel to the concentration of the protein.

The results of the above work, most of which was done on rabbits, might be attributed to the fact that the animals are herbivorous and not accustomed to the ingestion of large amounts of protein. Polvogt, McCollum, and Simmonds (4) in 1923 reported work done on the rat, an omnivorous animal, that tends to refute such a contention. Using high protein diets, they noted lesions in the kidneys that involved the glomeruli and tubules. Osborne, Mendel, and Cannon (5) in 1924 failed to confirm these results on the rat. In their work the weight of the kidney was about twice that of

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the kidney of the animal on a normal diet, but microscopic examination failed to disclose any abnormalities. Further work by Osborne, Mendel, Park, and Winternitz (6) substantiated these findings. Miller (7) also reported renal hypertrophy but no microscopic changes in rats' kidneys on high protein diets.

Confirmation of the earlier work was claimed by Evans and Risley (8) in 1925, who observed lesions in the kidneys of rats on diets high in protein content. Anderson (9), however, saw no lesions but did observe renal hypertrophy. Jackson and Riggs (10), and Smith and Möise (11) observed renal hypertrophy but no evidence of other damage on diets high in protein. MacKay, MacKay, and Addis (12, 13, 14, 15, 16, 17) have observed an increase in the size of the kidney as the amount of protein is increased in the diet or as cystine is added to the diet. On no occasion did they note any pathological changes in the kidneys when such diets were fed.

The question of whether renal lesions produced in this manner are due to the excretion of large amounts of the end products of protein metabolism, or to a toxic effect resulting from the failure of absorption products to be metabolized along their usual channels, has also been investigated. Newburgh and Marsh (18) in 1925 investigated the nephrotoxicity of several of the amino acids and found arginine, aspartic acid, histidine, lysine, tyrosine, tryptophane, and cystine to be injurious to the rabbit's kidneys. Curtis and Newburgh (19) and Lewis and Updegraff (20) also found cystine to be nephrotoxic.

Suggestion of a relationship between the amount of vitamin B in the diet and the effect of protein on the kidney was made by Drummond, Crowden, and Hill (21) in 1922. Reader and Drummond (22) also suggested such a relationship in the report of work which showed renal hypertrophy, but no histological changes, on high protein diets. Hartwell (23, 24) had definitely observed that an increase of protein in the diet necessitated that the vitamin B ration also be increased in order to enable the mother rat to raise a healthy litter. Reader and Drummond (25) again suggested such a relationship as a result of data obtained by them showing hypertrophy but no lesions of the kidney with diets high in protein but low in vitamin B. This condition disappeared when sufficient of the vitamin was fed. Still more convincing are the data of MacLean, Smith, and Urquhart (26) who were unable to produce signs of renal injury in rabbits with diets containing as much as 60 per cent protein as long as green food was given every day. When green food was withdrawn, renal injury developed as evidenced by albumin and casts in the urine. Further indication of such a relation-

ship was noted by Hassan and Drummond (27) and Hartwell (28), who observed that the protective factor resisted autoclaving.

Cox, Smythe, and Fishback (29) in 1929 observed definite tubular lesions in rats which had been subjected to diets containing 0.3, 0.6, and 0.9 per cent of cystine, respectively. This condition they diagnosed as "acute toxic nephrosis." Cox and Hudson (30) attempted to show a relation between the amount of vitamin B in the diet and this cystine nephrosis. They found that it was prevented by feeding 300 mg. of the Osborne and Wakeman yeast concentrate daily. They could find no relation between the development of nephrosis and the diet preceding the experimental period. They concluded also that the tendency to the development of the condition was an hereditary factor.

The controversial nature of the literature on this subject made it seem worthy of reinvestigation. The problem divides itself into three general questions. 1.—Do high concentrations of protein or cystine in the diet cause a definite pathological change in the kidney structure or, as some of the reports in the literature state, only an hypertrophy of that organ? 2.—Can the development of renal lesions or the hypertrophy, whichever occurs, be prevented or diminished in intensity by the use of whole yeast or yeast extract? 3.—What factor of the yeast extract is responsible for the beneficial effects, if any, obtained? The present paper is a report of an investigation of the first two questions.

Experimental

Male albino rats weighing between 30 and 65 grams at the beginning of the experiment were used. Such young rats are susceptible to cystine nephrosis, according to Cox and Hudson (30). The length of the experimental period was varied with the different groups as noted in the tables. The basal diet of Sherman and Spohn (31) was used and substitutions of cystine were made in this diet for the experimental groups.[1] Whole yeast extract was used as the source of the prophylactic agent. This extract was prepared by a method essentially the same as that described by Williams and Lewis (32).

The animals were kept in individual cages of the type used by Osborne and Mendel as described by Ferry (33). They were fed and weighed at regular intervals. Food and water were allowed *ad libitum*.

At the end of the experimental period the animals were weighed, killed

[1] In the preliminary work cystine was substituted for casein in the diet; in the experiments reported here in detail the cystine was substituted for corn starch.

by an occipital blow, and a complete autopsy was performed to ascertain if there was any gross abnormality that might be due to other causes than the effect of the diet. The kidneys were removed as quickly as possible, examined grossly, and all extraneous fat or other tissue was removed. The capsule was stripped off and the kidney sectioned longitudinally with a sharp instrument. A thin strip was made for tissue section, a cut being made on each side of the pelvis. This section was not more than 4 mm. thick and had a cut surface on each side so that fixation might take place as quickly as possible. Both kidneys were then immersed in a tared amount of Zenker's fixative in a glass stoppered bottle and weighed. The time that elapsed from the moment the kidneys were removed until they were placed in the fixing solution was never more than three minutes. Under these conditions post mortem autolysis was reduced to a minumum. After 12 to 24 hours fixation the kidneys were removed from the solution and sectioned for microscopic examination.

Comparison of the kidney weights was made with reference to the tables of Donaldson (34) and also by a study of the ratios between kidney weight and body weight. Hereafter, this ratio will be referred to as the K:B ratio.

Results

The results of kidney weight determinations for the first series of animals are given in Tables I and II. Comparison of Group I (basal diet) and Group II (basal diet and yeast extract), both on the basis of the K:B ratios and of the per cent deviation from normal kidney weights (Donaldson), shows that the vitamin B complex or some of its factors influences the development of the kidney. "Hypertrophy" of the kidneys appeared in those animals which did not receive yeast as a dietary supplement.

Groups III and IV, which received 0.3 per cent cystine, also show a marked difference in the size of the kidney in comparison to body weight. The kidneys of the animals of Group IV, which received whole yeast extract, were much smaller in comparison to body weight than those of Group III. Attention should be called to the fact that the comparative kidney sizes of Groups I and III are practically the same by both methods of calculation. This does not give to cystine at a level of 0.3 per cent in the diet any greater effect in the production of renal "hypertrophy" than the mere absence of the vitamin B complex from the diet.

Groups V and VI, which received a diet containing 0.6 per cent cystine, show a marked difference in comparative kidney weights. Seemingly, this is evidence that cystine at a level of 0.6 per cent in the diet does cause marked renal "hypertrophy" which is prevented by the addition of two

TABLE I

EXPERIMENTS SHOWING THE EFFECT OF CYSTINE DIETS ON THE SIZE OF THE KIDNEY WITH AND WITHOUT THE VITAMIN B COMPLEX

Group	Rat No.	Experimental period (days)	Cystine (per cent) *	Yeast Extract *	Initial weight (gm.)	Final weight (gm.)	Kidney weight (gm.)	K:B ratio	Per cent deviation from normal (Donaldson)
I	37	21	0	0	35.0	36.0	0.626	0.017	+45.8
	38	21	0	0	34.1	40.7	0.720	0.018	+77.4
	39	21	0	0	36.2	44.1	0.632	0.014	+26.5
	40	21	0	0	35.8	35.0	0.527	0.015	+25.4
	Average				*35.3*	*38.9*	*0.626*	*0.016*	*+43.0*
II	41	21	0	2.0	24.1	66.2	0.800	0.012	+17.1
	42	21	0	2.0	34.1	76.4	0.891	0.012	+16.4
	43	21	0	2.0	30.0	82.7	0.908	0.011	+11.4
	44	21	0	2.0	34.0	80.3	0.838	0.010	+17.9
	Average				*30.5*	*76.4*	*0.859*	*0.011*	*+15.7*
III	45	23	0.3	0	51.7	51.3	0.676	0.013	+20.5
	46	23	0.3	0	50.8	55.6	0.860	0.016	+44.2
	47	21	0.3	0	43.7	31.5	0.663	0.021	+71.3
	48	23	0.3	0	48.5	37.4	0.781	0.021	+77.2
	49	23	0.3	0	46.1	54.4	0.687	0.013	+17.3
	Average				*48.1*	*46.04*	*0.733*	*0.017*	*+46.1*
IV	50	23	0.3	2.0	41.0	101.7	0.956	0.009	+ 5.7
	51	23	0.3	2.0	44.6	104.0	0.954	0.009	+ 6.1
	52	23	0.3	2.0	44.6	105.1	1.021	0.010	+ 6.3
	53	23	0.3	2.0	46.0	109.7	1.053	0.010	+ 2.8
	54	23	0.3	2.0	42.2	95.7	0.913	0.010	− 0.5
	Average				*43.68*	*103.25*	*0.979*	*0.010*	*+ 4.1*
V	55	22	0.6	0	41.6	30.0	0.663	0.019	+57.9
	56	22	0.6	0	40.5	35.5	0.706	0.020	+66.4
	57	22	0.6	0	36.8	37.4	0.708	0.019	+60.6
	58	22	0.6	0	44.5	36.8	0.711	0.019	+63.2
	59	22	0.6	0	41.7	29.1	0.754	0.026	+106.2
	Average				*41.02*	*34.76*	*0.708*	*0.021*	*+70.8*

* In these diets the cystine replaced an equivalent amount of corn starch. Whole yeast extract was fed daily in a definite amount expressed in this table as gram yeast equivalents.

TABLE I—(*Continued*)

Group	Rat No.	Experimental period (days)	Cystine (per cent) *	Yeast Extract *	Initial weight (gm.)	Final weight (gm.)	Kidney weight (gm.)	K:B ratio	Per cent deviation from normal. (Donaldson)
VI	60	22	0.6	2.0	35.9	98.5	1.126	0.011	+20.0
	61	22	0.6	2.0	43.0	94.5	1.038	0.011	+14.6
	62	22	0.6	2.0	42.0	94.2	0.998	0.011	+10.3
	63	22	0.6	2.0	38.8	100.5	1.002	0.010	+ 5.1
	64	22	0.6	2.0	42.0	94.1	0.997	0.011	+10.3
	Average				*40.34*	*96.36*	*1.032*	*0.011*	*+12.1*

TABLE II

EXPERIMENTS SHOWING THE EFFECT OF HIGH PROTEIN DIETS ON THE SIZE OF THE KIDNEY WITH AND WITHOUT THE VITAMIN B COMPLEX

Group	Rat No.	Experimental period (days)	Casein (per cent) *	Yeast Extract *	Initial weight (gm.)	Final weight (gm.)	Kidney weight (gm.)	K:B ratio	Per cent deviation from normal. (Donaldson)
VII	65	24	40.0	0	42.0	33.6	0.718	0.021	+76.8
	66	24	40.0	0	57.5	54.2	0.947	0.018	+62.2
	67	24	40.0	0	58.6	56.1	0.917	0.016	+52.7
	68	24	40.0	0	41.5	44.0	0.695	0.016	+39.2
	69	24	40.0	0	38.1	40.0	0.652	0.016	+40.5
	70	24	40.0	0	42.7	40.5	0.823	0.020	+75.6
Average					*46.7*	*44.7*	*0.792*	*0.018*	*+57.8*
VIII	71	24	40.0	2.0	64.7	125.0	1.500	0.012	+31.2
	72	24	40.0	2.0	50.0	121.5	1.236	0.010	+10.8
	73	24	40.0	2.0	60.0	136.7	1.492	0.011	+21.2
	74	24	40.0	2.0	61.3	138.8	1.473	0.011	+18.1
	75	24	40.0	2.0	44.0	112.8	1.171	0.010	+10.3
Average					*56.0*	*126.9*	*1.374*	*0.011*	*+18.3*

* Whole yeast extract was fed daily in a definite amount expressed in this table as gram yeast equivalents.

gram yeast equivalents[2] per day of whole yeast extract prepared as noted above.

Groups VII and VIII (Table II), receiving 40.0 per cent casein in the diet, also show a marked difference in comparative renal weights. Group VII, which received no yeast extract, shows a 57.8 per cent increase over stated normals, a value which is not as high as that for Group V but distinctly higher than that of Groups I and III. Renal "hypertrophy" produced by a diet containing 40.0 per cent casein and prevented by the addition of whole yeast extract is indicated by these results.

It is worthy of note that the K:B ratio values for Groups II, IV, VI, and VIII, which received yeast extract, agree closely and are distinctly lower than the values for Groups I, III, V, and VII, which received no yeast extract. This demonstrates that the presence of the vitamin B complex, or some constituent of it, in the diet of the growing rat is necessary for the normal development of the kidney and that the kidney becomes larger when this necessary substance is lacking in the diet, the degree of "hypertrophy" depending upon other factors in the diet.

Microscopic examination of the kidneys of the animals which received casein showed normal renal structure in every instance. Of those which received cystine, rat 47 of Group III, on a diet containing 0.3 per cent cystine, developed a degenerative lesion in the tubular epithelium of both kidneys. The animal died two days before the end of the experimental period from this or some other undetermined condition. The kidneys of the remaining animals which received cystine were absolutely normal microscopically.

Table III gives the data for a second series of rats, all of whom received a diet containing 0.6 per cent of cystine. The dosage of yeast extract was varied in the different groups by increasing the yeast extract administration by 0.5 gram yeast equivalents in each successive group. This procedure was planned with the idea of determining the minimal dose of yeast extract which would be effective in overcoming the "hypertrophy" caused by 0.6 per cent cystine and to see if subminimal doses showed a graded response as the dosage was increased.

By comparison of both the K:B ratios and the per cent deviation from normal kidney weights (Donaldson), the results show that there was a graded response from Group IX (no yeast) to Group XII (1.5 gm. yeast equivalents). The kidneys of Groups XIII and XIV (2.0 and 2.5 gm. yeast

[2] The gram yeast equivalent is that amount of yeast extract which is obtained from the extraction of 1 gram of dry whole yeast. (Williams and Lewis, *Jour. Biol. Chem.*, 1930, 89, 275.)

TABLE III

EXPERIMENTS SHOWING THE EFFECT OF VARIED AMOUNTS OF VITAMIN B COMPLEX IN OFFSETTING RENAL "HYPERTROPHY" PRODUCED BY 0.6 PER CENT CYSTINE IN THE DIET

Group	Rat No.	Experimental period (days)	Cystine (per cent) *	Yeast Extract *	Initial weight (gm.)	Final weight (gm.)	Kidney weight (gm.)	K:B ratio	Per cent deviation from normal. (Donaldson)
IX	76	30	0.6	0	39.5	51.0	0.752	0.015	+34.9
	77	30	0.6	0	47.5	75.0	0.835	0.011	+10.9
	78	30	0.6	0	48.7	52.5	0.700	0.013	+22.9
	79	30	0.6	0	51.5	61.8	0.902	0.015	+39.5
	80	30	0.6	0	53.0	58.0	0.657	0.011	+ 6.7
	Average				*48.04*	*59.66*	*0.769*	*0.013*	*+22.98*
X	81	30	0.6	0.5	48.0	64.5	0.821	0.013	+22.7
	82†	30	0.6	0.5	47.5	62.0	0.924	0.015	+42.5
	83	30	0.6	0.5	48.5	71.0	0.803	0.011	+11.3
	84	30	0.6	0.5	40.0	59.5	0.630	0.011	+ 0.3
	85	30	0.6	0.5	45.0	70.2	0.938	0.013	+31.1
	Average				*45.8*	*65.44*	*0.823*	*0.013*	*+21.58*
XI	86	31	0.6	1.0	39.5	76.5	0.710	0.009	− 7.3
	87	31	0.6	1.0	43.6	84.0	0.787	0.009	− 4.6
	88	31	0.6	1.0	40.8	76.5	0.793	0.010	+ 3.6
	89	31	0.6	1.0	37.8	72.5	0.789	0.011	+ 7.6
	90†	31	0.6	1.0	45.0	90.0	1.221	0.014	+40.1
	Average				*41.34*	*79.9*	*0.860*	*0.011*	*+ 7.88*
XII	91†	31	0.6	1.5	50.0	96.4	1.021	0.011	+10.7
	92	31	0.6	1.5	44.0	97.5	0.849	0.009	− 8.8
	93	31	0.6	1.5	48.5	102.5	0.948	0.009	− 2.2
	94	31	0.6	1.5	44.0	102.2	0.934	0.009	− 3.3
	95	31	0.6	1.5	49.0	96.8	1.059	0.011	+14.4
	Average				*47.1*	*99.08*	*0.962*	*0.010*	*+ 2.16*

* In these diets the cystine replaced an equivalent amount of corn starch. Whole yeast extract was fed daily in a definite amount expressed in this table as gram yeast equivalents.

† Each of these animals had one fluid filled kidney. The weight of the kidney was taken after this retained fluid had been allowed to flow out.

TABLE III—(*Continued*)

Group	Rat No.	Experimental period (days)	Cystine (per cent) *	Yeast Extract *	Initial weight (gm.)	Final weight (gm.)	Kidney weight (gm.)	K:B ratio	Per cent deviation from normal (Donaldson)
XIII	96	32	0.6	2.0	41.4	94.5	0.996	0.011	+ 9.8
	97	32	0.6	2.0	44.5	116.0	0.990	0.009	− 7.8
	98	32	0.6	2.0	43.8	117.0	1.232	0.011	+13.8
	99	32	0.6	2.0	42.5	113.5	1.213	0.011	+15.1
	100	32	0.6	2.0	41.0	109.3	1.015	0.009	− 0.7
	Average				*42.56*	*110.06*	*1.089*	*0.010*	*+ 6.04*
XIV	101	32	0.6	2.5	43.2	116.3	1.128	0.010	+ 4.7
	102	32	0.6	2.5	40.5	91.0	0.967	0.011	+ 9.9
	104	32	0.6	2.5	47.0	108.9	1.068	0.010	+ 4.9
	105†	32	0.6	2.5	44.3	121.6	1.338	0.011	+19.8
	Average				*43.75*	*109.7*	*1.125*	*0.011*	*+ 7.86*

equivalents, respectively) show a slight increase above Group XII when compared to the Donaldson standards but very little difference in the K:B ratios. Evidently the minimal dose of the whole yeast extract used in this work that is necessary to prevent kidney "hypertrophy" when the diet contains a 0.6 per cent concentration of cystine lies somewhere near 1.5 gram yeast equivalents per rat per day.

Periodic examinations of the urine were made on the animals of these last six groups. The rats were placed in metabolism cages and the urine was collected under toluene at approximately 24 hour periods. At no time were there any casts, pus cells, erythrocytes, or amorphous sediment in the urine. There was at all times a slight albuminuria which did not increase during the course of the experiment. This was no more than is normal for the male rat.

Examination of the kidneys of these animals at autopsy revealed a gross abnormality resembling hydronephrosis in one kidney of each of four animals of the twenty-nine examined. Rat 82 had the right kidney involved and rats 90, 91, and 105 showed the condition in the left kidney. In each case the kidney contained a large quantity of yellow fluid which distended the organ to twice its normal size. Microscopic examination of the involved kidney showed that the medullary tissue had undergone pressure atrophy due to the presence of the retained fluid and, as a consequence,

was much thinner than normal. However, the remaining medullary tissue and the cortical tissue, as well as the tissue of the uninvolved kidneys, were entirely normal in appearance. Examination of the kidneys of the other twenty-five animals in these six groups revealed no abnormalities.

Discussion

Our experimental data show that both cystine (at a level of 0.6 per cent of the diet) and casein (at a level of 40 per cent of the diet) cause an enlargement of the kidney when whole yeast extract is not given as a dietary supplement. The administration of whole yeast extract prevented such an enlargement. Graded doses of yeast extract appear to have a graded action upon the size of the kidney as compared to body weight. This gives to some component of yeast, probably vitamin B complex or one of its factors, the property of preventing the increase in size resulting from the action of cystine or excess casein in the diet. These findings agree with the reports in the literature previously cited (25, 26, 27, 28, 30).

Quotation marks have been used advisedly in this report when speaking of "hypertrophy." Whether it is a true, definite, organ hypertrophy, which would persist if the causative factors were removed, or whether it is merely a work enlargement, which would disappear with removal of the cause, was not determined.

Notable throughout this work is the *complete absence* of evidence that a diet high in protein or cystine produces pathological lesions in the kidneys. Regardless of the type of diet, there was no evidence of degenerative kidney lesions with the single exception noted above (rat 47, Group III). Since this condition developed in this one individual only, it is improbable that it was due to the cystine in the diet. Our failure to produce kidney lesions is contrary to the observations of Cox, Smythe, and Fishback (29) that "cystine nephrosis" develops in rats fed cystine during a short experimental period. In our experiments with casein, a 40 per cent concentration of this protein was in no way nephrotoxic. It should be noted that our experimental period was probably much too short for the development of renal lesions, since Newburgh and Johnston (35) have reported that many months on a high casein diet are required to produce pathological changes in the kidney.

Further evidence that cystine is not nephrotoxic is given by the results obtained in some preliminary work done before the experiments reported above. Rats were given the basal diet used throughout our work plus 0.3 per cent cystine, an amount which had been found by Cox, Smythe, and Fishback (29) to be nephrotoxic to rats. The animals used were from a local

supply source and were in poor nutritive condition when received. Their general state of health was so poor throughout the experimental period that no attempt was made to draw conclusions from the data obtained concerning the effect of the diet on kidney weight. However, it is interesting to note that microscopic examination of the kidney tissue gave no evidence of pathological changes. Positive pathological findings in this group of animals would have had no weight, since animals in a poor nutritive condition might be expected to have a greater tendency than more healthy animals for the development of pathological lesions. The negative findings which were observed, however, seem most significant.

As stated previously, this work was begun with the intention of identifying the factor present in the water soluble portion of yeast that is responsible for its activity in the prevention of cystine nephrosis. The consistently negative tissue findings encountered in the early part of the work necessitated the development of the problem along altogether unexpected lines. Further work on the identification of the factor or factors of yeast extract responsible for the prevention of renal hypertrophy caused by cystine is now in progress in this laboratory and will be reported at an early date.

Conclusions

1. Vitamin B complex in the diet prevents abnormal development of the kidney of the rat under the influence of cystine or excess casein.

2. Cystine, 0.3 per cent, in the diet does not cause renal "hypertrophy" to any greater degree than the mere absence of yeast extract from the otherwise normal diet. A concentration of 0.6 per cent cystine, however, does cause an "hypertrophy" which may be prevented by the administration of vitamin B complex.

3. Excess casein in the diet likewise causes renal "hypertrophy" which is also prevented by the administration of vitamin B complex.

4. Graded doses of whole yeast extract cause a progressively decreasing response on the part of the kidney to the administration of cystine. This is true up to a certain point in the vitamin dosage. Increase above that point does not have any further beneficial effect.

5. Cystine was *not nephrotoxic* to these animals in the sense of causing degenerative lesions of the kidney.

The tissue sections used in this work were examined by Dr. W. C. Johnson, Head of the Department of Pathology, who confirmed our findings. The authors wish to express thanks to Dr. Johnson for his kindly advice and suggestions during the course of this problem and to the technical

staff of his department for their help in the preparation of tissues for microscopic examination.

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THE METABOLISM IN PREGNANCY
IX. THE FOETAL INFLUENCE ON THE BASAL RATE*

By

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IN A SERIES of earlier papers the senior author and his associates (1) have discussed the results of extended investigation on certain aspects of the metabolism in normal pregnancy. Subjects for this study were drawn both from an out-patient prenatal service (Series "A") and from two maternity institutions (Series "B" and "C") in order to secure a group truly representative of the conditions of practice. All studies were continuous from the time of initial contact through the completion of the pregnancy and for a number of weeks beyond, an experimental condition that added materially to the investigative difficulties but increased proportionally the significance of the data secured. Only those patients were retained who demonstrated complete normalcy of function throughout the period of observation. As all of the descriptive data have already been presented in the earlier papers, only a few of the fundamental records need be repeated here for purposes of orientation.

TABLE I

FUNDAMENTAL DATA

Datum	Series			Total or average
	A	B	C	
Number of cases	25	21	31	77
Age, (years)	28	18	19	22
Height, (cm.)	158.8	160.4	160.2	159.8
Weight,† (kg.)	67.0	64.4	66.8	66.3
Weight increase, kg. per week	+0.32	+0.52	+0.54	+0.46
Weight of baby, (kg.)	3.31	3.20	3.28	3.27
Interval of study				
Maximum	34 weeks	13 weeks	25 weeks	
Average	21 "	8 "	14 "	

† 1 week ante-partum.

* Presented before the American Physiological Society, Montreal, April 1931.

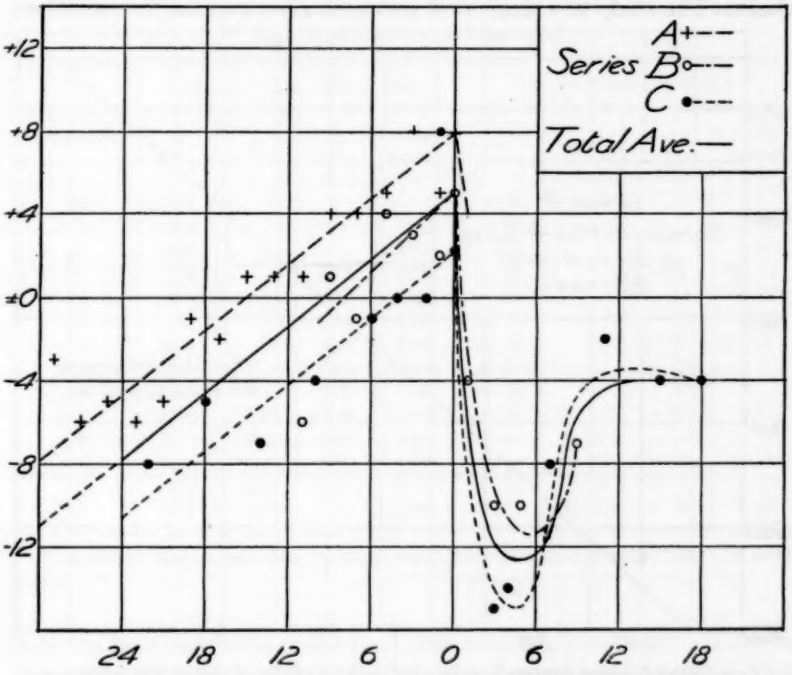
But minor comment is necessary. The youth of the group was determined largely by the sources of Series "B" and "C." Comparison of the data of these series with those of an older group studied in another connection and not included here shows that the present figures are wholly representative. The weights are given one week ante-partum as there is some evidence to support the contention that a loss of weight occurs in the few days immediately before delivery. The point is not wholly established, however, and the present convention avoids uncertainty.

The interval of study was naturally determined by the time of initial contact. In Series "B," admissions to the home were usually in the later months of gestation and the duration of the study was correspondingly circumscribed. The average duration in both Series "A" and "C" is unduly affected by the inclusion, for other purposes of the whole investigation, of a few cases who were first seen as the pregnancy was approaching termination. Adequate data are available to construct wholly representative curves for Series "A" and "C" over intervals of 27 and 21 weeks respectively.

A highly interesting and important datum is found in the estimation of the energy requirement as measured by the respiratory exchange (1, b, f). The results can be presented most compactly in graphic form. To absorb the differences produced by changing weights the deviations from prediction rather than the actual observed rates are plotted, thus indicating the relative changes. Following the practice of this institution, comparisons are made with the standards both of Harris and Benedict (2) and Aub and DuBois (3) and the deviation from their mean recorded. With the exception of Series "B", where the difference approximates 6 per cent, the variation between the predictions by the two formulas is 3 per cent or less.

The curves from the three independent series give entirely consistent results, each showing a linear upward change during the progress of the pregnancy; the slopes are substantially identical—exactly so in the case of the two longer and thus more reliable periods of study. The absolute difference of 6 per cent between these two extreme curves is readily explained by the varying conditions of testing. Series "A" was drawn from an outpatient service and the subjects came from their homes to the hospital on the morning of the measurement. While every effort was made to insure basal conditions before testing by the usual resting procedure, these subjects were patently less basal than were the patients constituting Series "C." With these latter, all of whom were hospital inmates, the machine was carried to the bedside and in many instances the patient was aroused

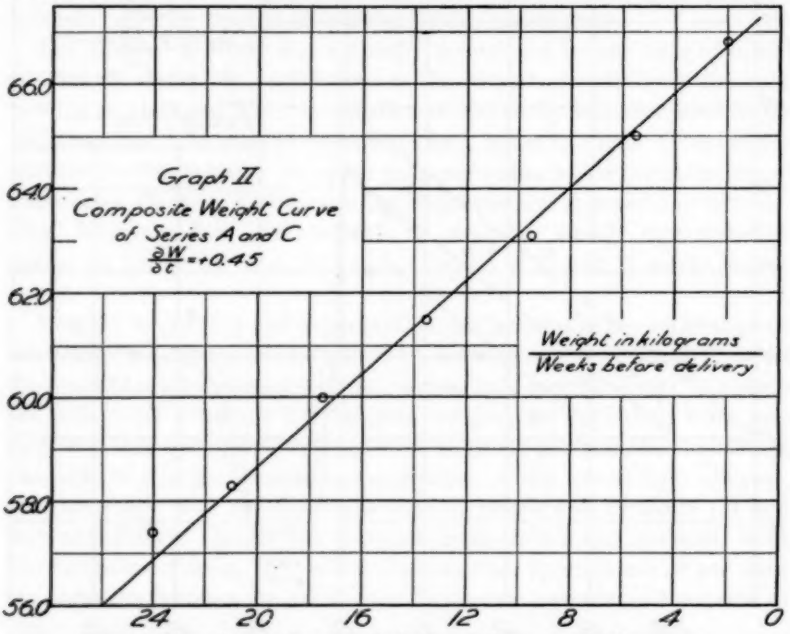
from the night's sleep in order to apply the test. The difference in the approach to basal conditions of the two groups is reflected in the observed rates. The solid line gives the average of the entire group and may be regarded as a representative portrayal of the changes occurring in pregnancy. It will be noted that the point of origin of each curve is below prediction and that the level at the time of delivery is superior to the cal-



GRAPH 1. Variations of observed from predicted Total Basal Metabolism for 24 hours during the last six lunar months of pregnancy. Prediction standard is the average of the Harris-Benedict (2) and Aub-DuBois (3) indications.

culated normal. This point will be discussed later in the body of the paper. Patently, during the last 24 weeks of gestation at least, there is a progressive increase in the total basal for 24 hours of the order of 13 per cent over and above that conditioned by the increase in weight. The pregnancy is the major factor of difference between these patients and a like series of women demonstrating a progressive obesity but in a state of sexual rest and to this special physiological condition one turns for an explanation of the increased energy requirement.

Some years ago, Sandiford and Wheeler (4) analyzed the data of a single patient studied throughout gestation. They reached the conclusion that the foetal metabolism, as correlated with the surface changes engendered by foetal growth, was adequate to account for the entire difference. More recently Boothby and Sandiford (5) have made a second study on the same patient in a later pregnancy and again have reached the same conclusion. The study of a single case makes no allowance for the variations



GRAPH 2. Linear increase in weight during last six lunar months of pregnancy.

recognized to occur normally in this magnitude—the conventional allowance of ±10 per cent in defining the zone of normal performance is an illustration. With so large a series as that under consideration and with the undoubted correlations between the component groups, it seems warrantable to assume that these present data may serve as the basis for a rigorous investigation of the validity of the conclusion given above. The present paper deals with such a survey. Studies of the individuals composing this group show that the maternal increase in weight, at least for the last 6 lunar months, is substantially linear (1, i). A composite of those cases in Series "A" and "C"—a significant majority—where the period of study was of adequate length may be used for illustration.

From the basic biometric magnitudes and the curves of deviation of the basal rates it is easy to calculate both the actual rates—from which the curves were initially derived—and those rates predicted by the two accepted standards. In this way, the actual deviations in terms of calories per 24 hours can be readily computed. The data are collected in the next table.

TABLE II

BASAL RATE VARIATIONS. (CALORIES)

Weeks Ante-partum	Observed Rates from Curves				Deviation from Prediction			
	A	B	C	Combined*	A	B	C	Combined*
27	1315				− 84			
25	1336			1272	− 69			−112
23	1358			1296	− 54			− 97
21	1379		1292	1320	− 39		−128	− 82
19	1400		1317	1344	− 24		−113	− 67
17	1422		1343	1368	− 9		− 98	− 52
15	1443		1369	1393	+ 6		− 83	− 37
13	1464		1395	1417	+ 21		− 68	− 22
11	1485		1420	1440	+ 36		− 53	− 7
9	1506	1477	1446	1464	+ 51	− 7	− 38	+ 8
7	1527	1506	1471	1488	+ 66	+ 11	− 23	+ 23
5	1548	1534	1497	1513	+ 81	+ 29	− 18	+ 38
3	1570	1564	1522	1537	+ 96	+ 48	+ 7	+ 53
1	1591	1593	1548	1560	+111	+ 67	+ 22	+ 68

* Calculated from the solid line in Graph 1 which represents the average of the entire study group.

In the table above the results under the caption "combined" are drawn from the average of the data of the complete group. Inspection shows that there is an increase in the energy requirement of between 7 and 8 calories per week in excess of that conditioned by the increment of the maternal weight. Series "B" shows a somewhat higher value but in this group the period of ante-partum study was relatively brief and thus the data lack the authority of those from the more protracted studies.

Experience has demonstrated that the surface area of the living organism is a biometric unit of great convenience as a standard of reference and the area, calculated from height and weight, is a fundamental factor in the widely used Aub-DuBois prediction formula.

Using the well-known Lissauer (7) formula, $S = 10.3W^{0.667}$, Sandiford (6) calculated foetal areas and these were used as the basis of calculation in

the two papers (4, 5) already mentioned. In a long series of biometric studies by Scammon and his associates, one publication by Klein and Scammon (8) gives the results of a series of actual measurements of foetal surface and derives formulae based upon these observations for the calculation of the area from the observed crown-rump and crown-heel heights and from the observed weight. In a later paper the same authors (9) present additional evidence of the applicability of these formulae. Scammon's measurements were made upon the dead foetus and he with Calkins (10) had previously noted a difference of 220 gms. between the weight of the live child and that of the dead foetus. Desiccation with loss of weight obviously takes place, a fact which was quantitatively recorded by Ipsen (11) in 1894. In Scammon's table the weight at birth is recorded as 3.00 kgm. which would correspond to a weight of 3.22 kgm. for the living child. This value, fortuitously, is practically the same as the averages of our several series (see Table I). Loss of weight by desiccation will entail a diminution of surface area so that a correction must be made for weight differences if living areas are to be calculated by the Scammon weight formula, $S = 5.188\ W^{0.75}$. We have taken Scammon's actual weight records and computed the foetal areas at different periods of development by use of the formula given above and then made suitable correction for the weight differences between our own mean birth weights and that of Klein and Scammon,[1] (Columns 3, 4, 5, 6 of Table III). Further, we have calculated the foetal weights from our surfaces by the Klein and Scammon weight-area relation (based upon actual measurement) and to these values have applied the Lissauer surface formula (Column 7, Table III). Finally, we have reviewed the surface areas computed by this formula by Boothby and Sandiford (5) from weights which they derived by computation (Column 8, Table III) and have also performed a similar review of the values recorded by Sandiford and Wheeler (4) (Column 9, Table III). In this last series (Column 9) the final weight was estimated by the authors as 3.90 kgm. and the surface computations were based by them upon this with the proper decrements for the preceding time intervals. The actual weight of the child as delivered was 3.60 kgm. and the recorded surface values are consequently somewhat too high. The results of the several series of computations are collected in the following table.

The agreement of the results in our own several series is to be anticipated as the uniformity of the data from which they are derived ensures this

[1] The Scammon equation is written Surface = 5.188 (Weight)$^{0.75}$. Our weight can be taken as R×W where R = the ratio between live and dead weights. The maximum differences introduced by this correction are of the order of 8 per cent or less.

TABLE III

FOETAL AREAS. (square meters)

1	2	3	4	5	6	7	8	9
Weeks	Scammon & Klein original data	Modified Scammon Formula (R & B Data)				Lissauer Surface from		
		Ser. A	Ser. B	Ser. C	Average	Wt.(Scammon)	Wt.(B&S)	Wt.(S&W)
28	0.004	0.004	—	—	0.004	—	—	—
24	.015	.016	—	0.016	.016	0.021	0.029	0.040
20	.034	.036	—	.036	.036	.042	.049	.050
16	.059	.064	0.62	.063	.063	.071	.075	.080
12	.090	.097	.95	.096	.096	.101	.105	—
8	.126	.136	.133	.135	.135	.136	.140	.150
4	.166	.179	.175	.178	.178	.174	.185	.200
0	.210	.226	.221	.224	.224	.214	.230	.260
Child (Weight)	3.00	3.31	3.20	3.28	3.27	3.00	3.30	3.90*

* Estimated by Sandiford & Wheeler and used in the computations. Actual weight = 3.60 kg.

result. Equally, the agreement with Scammon's actual observations is very close and for the same reason this was to be anticipated as we use his formula based on his own actual measurements. Less good, certainly in the earlier months, is the agreement with the Boothby and Sandiford calculations. At these periods, however, the foetus is very small and the addition which it makes a scarcely significant one (*v.i.*). As noted above, the Sandiford-Wheeler figures are too high and correction here would improve the correlation with the results from the Scammon formula but still leave the agreement poor in the earlier months.

The maternal areas at the several stages of gestation can be readily calculated from the maternal weights by means of the formula of DuBois and DuBois (12). We are inclined to question the convention adopted in the two papers under discussion, of subtracting the foetal from the maternal weight before computing the latter area. Certainly the increase in the pelvic and abdominal contents produces a definite increment in the surface area of the mother. The area calculated from total weight for the sake of equality will be used as the basis of the subsequent comparisons. This naturally will give slightly higher values for the maternal area than those calculated in the Boothby-Sandiford-Wheeler studies. In any case the difference is a minor one (a maximum of less than 3 per cent) and does not materially affect the final result of the next correlation.

The method of approach in the two papers under analysis was to calculate the maternal and foetal areas independently and combine them to express the area of the pregnant organism. In other words, by adding the foetal to the maternal surface, the total effective area is obtained and the heat production is referred to this as the biometric standard of comparison. The warrant for this approach may be questioned— and is by the present authors—as it is difficult to see how a selective heat loss could take place from a body under the presumably adiabatic conditions that obtain for the foetus in pregnancy. The foetal heat production, as we conceive it, would depend on the active mass of protoplasm—of which the surface is not an exact measure—and would ultimately be lost by conduction through and radiation from the maternal organism as is the energy from the viscera. The present thesis is to analyze the above assumption from the standpoint of its consistency with the actual data of observation.

Reverting to the exposition, by dividing what we may term the total effective area into the total caloric output for 24 hours, and dividing this result in turn by 24, the heat production per square meter of "effective area" per hour is secured. The results of this manipulation are given in the next table. To avoid an unnecessary multiplication of concordant figures,

comparisons are reported only with the data of Series "A" to serve as illustration and with those which are the average of the entire group (Referred to subsequently as "Total").

There is an approximate constancy to this final quantity, the range in the "A" series being 0.98 in 34 to 35 calories, while the combined group shows the slightly smaller and non-significant total variation of 0.78 calories in 33 to 34, a matter of less than 3 per cent. Recalculating the maternal complement by using the Boothby-Sandiford-Wheeler convention of de-

TABLE IV

HEAT PRODUCTION IN TERMS OF TOTAL EFFECTIVE SURFACE

Weeks	Area Mother and Foetus		Obs. B.M.R.		Heat Prod. per sq. meter	
	A	Total	A	Total	A	Total
27	1.604	—	1315	—	34.16	—
25	1.617	1.577	1336	1272	34.43	33.61
23	1.633	1.596	1358	1296	34.65	33.84
21	1.650	1.617	1379	1320	34.82	34.01
19	1.669	1.640	1400	1344	34.95	34.15
17	1.690	1.663	1422	1368	35.06	34.27
15	1.712	1.689	1443	1393	35.12	34.39
13	1.736	1.717	1464	1417	35.14	34.39
11	1.761	1.745	1485	1440	35.14	34.38
9	1.787	1.775	1506	1464	35.11	34.36
7	1.814	1.807	1527	1488	35.07	34.31
5	1.843	1.838	1548	1513	35.00	34.30
3	1.873	1.870	1570	1537	34.93	34.25
1	1.904	1.903	1591	1560	34.82	34.15

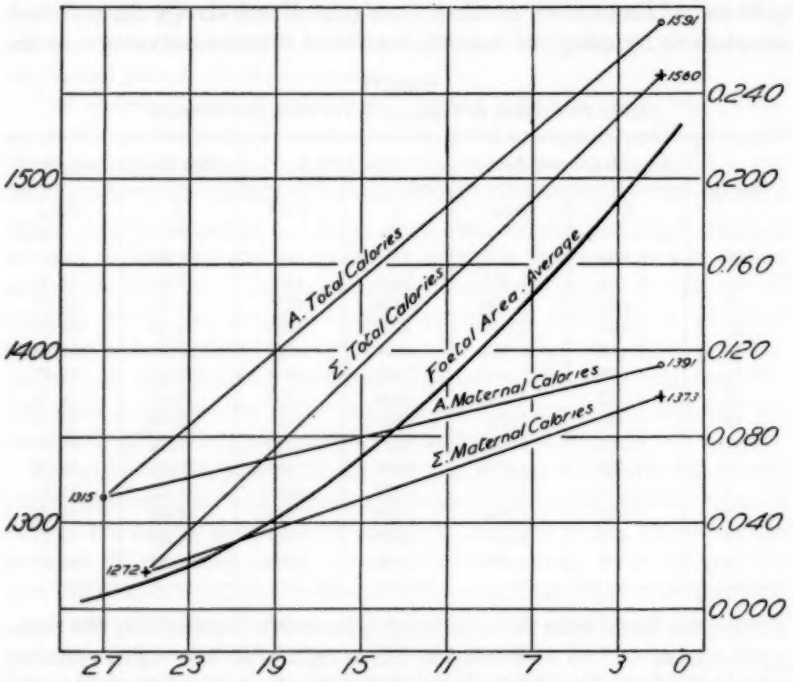
ducting the foetal from the total weight, the result is practically the same, 1.16 in from 33.5 to 34.5 calories. These figures, at first sight, certainly seem to confirm the conclusions reached in the two papers under discussion. It will be noted, however, that the values of the heat production per square meter show a definite progression defining a curvilinear relationship with a maximum approximately midway in the series.

A more rigorous analysis of the existing data can be secured through determining the excess heat production due to the foetus by deducting the maternal from the total caloric production and then comparing these values with the foetal area.[2] The underlying approach can be shown

[2] In calculating the maternal heat production, the assumption is made that at some selected time before delivery the foetal influence on the energy exchange, at first of necessity inconsiderable, makes itself manifest in tangible amount (See Graph V). From this point on, the maternal prediction is based on the total weight increases demonstrated by actual measurement. This

graphically by plotting the total and maternal output. The intercepted area is that which is ascribed presumably to the excess foetal production.

All of the existing data, both in these series and in the few others recorded in the literature, show a linear change in the relative basal rate. Equally, all of the computations of foetal area (see Table III) define a curvilinear relationship. Naturally any arithmetical adjustment of these



GRAPH 3. Total and maternal heat production with synchronous change in Foetal area. Curves marked "A" from Series "A," marked Σ from the "Total" group.

two quantities must determine a curvilinear change. The magnitude of the variation can best be demonstrated by the figures themselves. They have been computed for the several groups and the results for Series "A" and the "Total" group are assembled in the next table.

The variation here is of a very significant magnitude and certainly does

assumes that the total protoplasmic mass (based on the total weight) is related to the energy exchange of the pregnant woman as experience has shown it to be in the non-pregnant. The weight is the sole variable in both of the prediction formulae under present conditions. The warrant for this convention is discussed later in the text.

not imply any constant heat production by the foetus. Further, selecting any of the individual values of the foetal heat production and calculating the area changes necessary to give constancy to the output, a surface curve is defined that bears no relation to any of those based upon actual measurement as given in Table III. To illustrate, if the value for the foetal area, 0.106 at 11 weeks ante-partum in Series "A," be assumed to be correct, then the area at 21 weeks should be 0.40 and at one week 0.171. Any such attempted adjustment would but ill accord with facts and yield no more than an arithmetical artifact.

The method followed in calculating the maternal heat production from the gross measured maternal weight includes the weight of the foetus as well. The legitimacy of this procedure may well be questioned as it thus includes the weight prediction factor of the foetus when the Harris-Benedict formula is used. This gives too high a value for the maternal energy requirement and consequently, by difference, too small an amount for the foetal heat. The same objection does not hold when the Aub-DuBois generalization is used as there is an actual increase in the external surface of the mother as the uterus enlarges.

In order to estimate the order of magnitude of the error deriving from the inclusion of the foetal weight in predicting by the Harris-Benedict formula, the maternal contribution has been calculated after deduction of the weight of the foetus. The values thus obtained have been averaged with the uncorrected Aub-DuBois prediction. Further, that the story might be complete, the Aub-DuBois areas have been corrected by subtracting the foetal from the maternal weight before calculating the area, and the mean of the corrected[3] Aub-DuBois and corrected Harris-Benedict predictions compared with the values actually obtained. The results can be presented most compactly in graphic form.

At first sight, the greatest constancy would seem to obtain with the doubly corrected values. Even here, however, omitting the value at 23 weeks, the difference is 8.0 calories as against 10.2 for the wholly, and 8.8 for the partly, corrected predictions. Further, arbitrary manipulation of the foetal caloric output does not improve the situation unless the maternal contribution be assumed to follow a curvilinear course which compensates for that of the surface variation. For such arbitrary correction there is no experimental support. One other point to be noted is that with each deletion of the maternal heat production, that calculated for the foetus

[3] This is an over correction as it omits the actual increment in the maternal area deriving from the increase in the pelvic contents (*v.s.*).

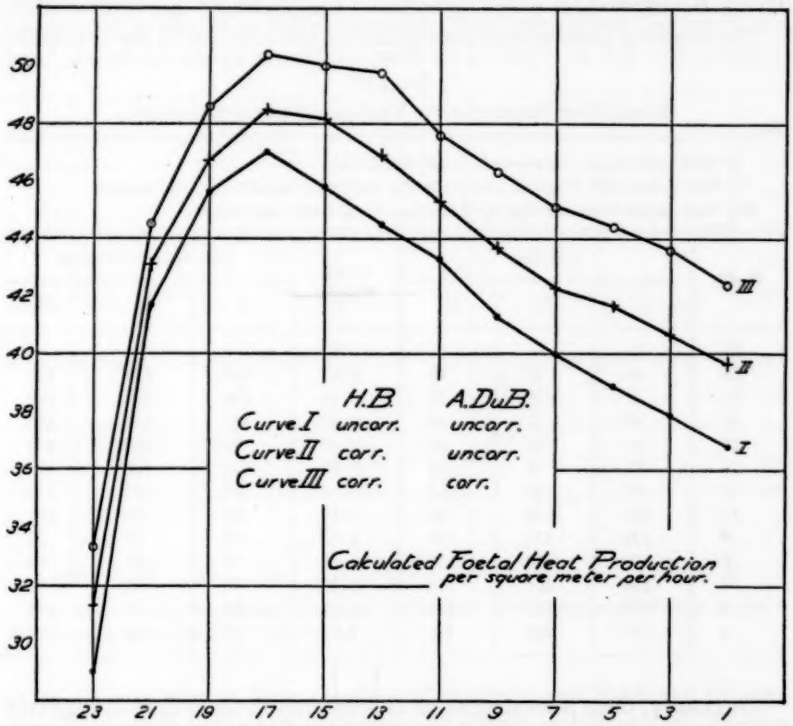
TABLE V

FOETAL HEAT PRODUCTION

Weeks	Total Cal. (obs.)		Maternal Cal.		Foetal Cal.		Foetal Area		Foetal Cal. per Sq. Meter per hour	
	A	"Total"	A	"Total"	A	"Total"	A	"Total"	A	"Total"
27	1315	—	1315	—	—	—	0.006	—	—	—
25	1336	1272	1320	1272	16	—	.012	0.012	55.56	—
23	1358	1296	1326	1282	32	14	.020	.020	66.67	29.17
21	1379	1320	1332	1290	47	30	.030	.030	65.28	41.67
19	1400	1344	1338	1298	62	46	.042	.042	61.51	45.64
17	1422	1368	1344	1306	78	62	.056	.055	58.04	46.97
15	1443	1393	1349	1316	94	77	.071	.070	55.17	45.83
13	1464	1417	1355	1324	109	93	.088	.087	51.61	44.54
11	1485	1440	1361	1331	124	109	.106	.105	48.74	43.25
9	1506	1464	1367	1340	139	124	.126	.125	45.97	41.33
7	1527	1488	1373	1348	154	140	.147	.146	43.65	39.95
5	1548	1513	1379	1357	169	156	.168	.167	41.92	38.92
3	1570	1537	1385	1365	185	172	.191	.189	40.36	37.92
1	1591	1560	1391	1373	200	187	.214	.212	38.94	36.75

lies progressively farther away from the actual amount determined by experiment both in the mother and in the new-born child.

The basal metabolism is potentially a measure of the active protoplasmic mass and body weight is a rough measure of this quantity even though it can be no more than a first approximation because of the di-



GRAPH 4. Comparison of calculated foetal heat production per square meter per hour, based upon application of correction of foetal weights to value of maternal weight used in computing maternal energy alone.

versity of the tissues involved. Calculating foetal weights by the Scammon equations, the heat production per kilogram per 24 hours is readily computed.

As partly explained above, three methods of calculation have been used. All assume a relative constancy in the maternal contribution from some point in the pregnancy as illustrated in the data of Table V. The first calculation approach (I) uses the gross maternal weight (observed) as the "W" in both the Harris-Benedict and Aub-DuBois prediction equations.

The second (II) deducts the foetal weight from the gross maternal weight to yield the arithmetical value for the "W" of Harris-Benedict but persists in the first convention as regards the Aub-DuBois "W." The third (III) and last uses this modified "W" (gross maternal minus foetal weight) for the area computation of the Aub-DuBois prediction as well as the modified Harris-Benedict value which appeared in the preceding (II) computation.

The results of these several manipulations are collected in the next table.

TABLE VI

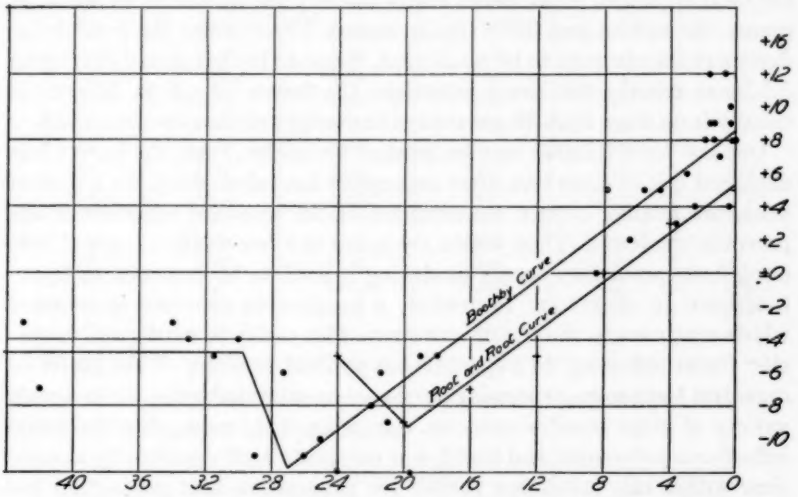
FOETAL HEAT PRODUCTION PER KILOGRAM COMBINED CURVE ONLY

I. Both predictions uncorrected. Total weight used.
II. Harris-Benedict Prediction corrected by deducting foetal from total weight.
III. Both predictions corrected by deducting foetal from total weight.

Weeks	Calories			Foetal Weight	Calories per kilogram		
	I	II	III		I	II	III
25	—	—	—	0.07	—	—	—
23	14	15	16	0.13	108	115	123
21	30	31	32	0.22	136	141	145
19	46	47	49	0.35	131	134	140
17	62	64	67	0.51	122	125	129
15	77	81	84	0.70	110	116	120
13	93	98	102	0.93	100	105	110
11	109	114	120	1.19	92	96	101
9	124	131	139	1.50	83	87	93
7	140	148	158	1.83	77	81	86
5	156	167	178	2.20	71	76	81
3	172	185	198	2.61	66	71	76
1	187	202	216	3.05	61	66	71

Obviously, there is no suggestion of constancy in the weight-energy relation. A similar computation for the maternal organism correlating the maternal heat production calculated from weight increase with and without correction for foetal weight shows a variation between the extreme values of less than 10 per cent. Further, the individual maternal value per kilogram one week before delivery is only about one-third of that calculated for the foetus per kilogram at the same interval. Benedict and Benedict (13) have shown that the influence of a bath at neutral (body) temperature is to raise the oxygen consumption approximately 10 per cent in the majority of cases. This is of a very different order from the nearly 200 per cent difference between maternal (environmental) and foetal heat production per kilogram as recorded above.

But one other question comes to immediate attention. Prior to conception the normal healthy woman should and does have an energy requirement closely in accord with prediction. After she conceives, all of the existing observations show that at some point fairly early in the pregnancy there is a drop in the oxygen requirement to a level which may exceed the conventional lower normal limit of −10 per cent. Further, subsequent to this drop there is a steady progressive linear rise reaching a level somewhat above prediction prior to delivery. In the next graph is



GRAPH 5. Progressive change in oxygen requirement during pregnancy showing period of readjustment of the gestating organism.

given a curve plotted from the data of Boothby and Sandiford (5) and also one from the observations reported by Root and Root (14), likewise on the study of a single case.

In the Boothby and Sandiford curve the inversion comes somewhere between the 30th and 26th week before delivery while the Root and Root depression seemingly occurs between the 24th and 20th week. In our own series fourteen of the patients were studied over periods of sufficient duration to permit a record of this period of transformation. In all of them it fell between the 30th and 22nd week before delivery, the estimated average mid point lying in the neighborhood of the 25th to 26th week. A full discussion of the implications of these observations is scarcely germane to the present text and will be considered elsewhere by one of us (15). While the

gestational period is highly variable and viable children may be delivered after pregnancies ranging from 33 to 47 weeks, the great majority terminate after an apparent span of 38 to 39 weeks. On the basis of the averages, the period of involution falls between the middle of the 3rd and that of the 4th lunar months. While one may concede the possibility of an active change in hormone control or other agency during the earlier weeks, it is hard to believe that the tiny protoplasmic mass of the foetus *per se* can exercise any very significant influence on the energy requirement of the total organism. At 27 weeks before delivery the estimated weight is 30 grams, the surface area 0.006 square meters. There is also the possible influence of the placenta to be considered. Benoist (16) has stated that up to 3.5 lunar months this tissue outweighs the foetus though at delivery it usually is no more than 20 per cent of the weight of the new-born child.

One last consideration may be touched on briefly. From the curves just discussed it is obvious that after conception has taken place, for a time at least, the relative oxygen consumption of the maternal organism is apparently unaffected. Then within the space of a few weeks a marked lowering from prediction occurs producing a level to be regarded as hypofunctional in character. Thereafter, a progressive recovery is manifest which continues to the end of gestation. This could be attributed to specific foetal influence, to a spontaneous gradual recovery of the maternal organism from some depressing hormonal or other influence, or to a wide variety of other possible agencies. Assuming, once more, that the foetal influence is paramount and that it first manifests itself significantly at some time within this involution period, the progressive heat production can be readily computed from a series of intermediate steps, each assumed as the point of initial foetal influence. To compute this, the total metabolism as actually observed is taken as the starting point. The maternal contribution is then calculated from the weight increase, using the Harris-Benedict prediction with foetal weight deducted and Aub-DuBois prediction from area calculated from total weight, in other words, uncorrected for the foetus. If, for example, the observed basal rate of the mother is 10 per cent below prediction twenty-nine weeks before delivery, it is assumed that she maintains this relation throughout the remainder of the gestation. The total foetal contribution can then be computed by deducting the maternal metabolism as calculated from the total metabolism as observed. This difference, which is the assumed foetal contribution, is then correlated with the foetal area and the value per square meter computed. Such a series of data is collected in the next table.

Again there is a real lack of such constancy as might be anticipated were

TABLE VII

CALORIES FROM FOETUS ALONE CALCULATED ON BASIS OF HEAT PRODUCTION PER SQ. METER OF FOETAL AREA PER 24 HOURS

MATERNAL CONTRIBUTION CALCULATED USING HARRIS-BENEDICT CORRECTED AND AUB-DUBOIS UNCORRECTED PREDICTION VALUES

Combined Groups

Weeks	29	27	25	23	21
29	0	—	—	—	—
27	3000	0	—	—	—
25	2833	1333	0	—	—
23	2550	1600	750	0	—
21	2267	1600	1033	567	0
19	2025	1500	1119	762	381
17	1855	1455	1164	890	600
15	1714	1414	1157	971	729
13	1575	1334	1127	966	782
11	1457	1248	1086	943	790
9	1380	1192	1048	928	800
7	1280	1137	1013	918	801
5	1227	1107	994	916	814
3	1175	1073	979	899	809
1	1122	1038	955	882	802

the foetal metabolism the sole or even the primary factor in producing the demonstrated excess heat production.

DISCUSSION

Adding the foetal area to that of the mother reduces the variation in the heat production per square meter in the last 6 months from 13 per cent to about 3 per cent (see Table IV). On this basis it has been concluded that the increasing foetal metabolism is the factor determining the apparent increasing over-production of energy when the calculation is based upon the maternal organism alone. But if the arithmetical processes involved be considered, it will be found that a small, variable, progressive, corrective factor has been added to a much larger quantity which is also showing increase but at a slower rate. Any other biometric entity showing a similar change could be manipulated to give equally concordant results. For example, one of us (17) has shown that the weight of the average individual can be calculated from the hip circumference with a very fair degree of precision. When the formula is applied to the pregnant woman, at least in the later months of gestation, it gives values from 5 per cent to 8 per cent too low as the pelvic contents represents a localized weight increment not

affecting the hip girth. By a suitable arithmetical manipulation of this minor variable (i.e., increasing hip girth) as a corrective factor a relatively uniform heat production throughout gestation could be apparently demonstrated. This is, of course, purely artifactual; the later analyses in this paper of the foetal influence would seem to indicate that the apparent constancy of the heat production in terms of the combined areas derives from a similar origin. Further, the normal heat production of the maternal organism with the age range of this series would be 37 to 38 calories per square meter per hour (3). The figures actually obtained are from 33 to 35 calories. The heat production of the new-born as measured by Benedict and Talbot (18) ranges from 27 to 36 calories. With the relatively small contribution made by the foetus to the combined surface, the foetal production would have to be of the order of 10 to 15 calories per square meter per hour one week before birth to account for the values recorded in Table IV if maternal consumption were normal. To conclude then, the deduction that the excess increment in the respiratory metabolism during the last 6 lunar months of pregnancy is due solely to the foetal metabolism would seem not to be warranted in fact. That the foetal metabolism plays its part no one could deny, but the special activity of this particular tissue mass is absorbed, in part at least, by the biometric magnitudes used as correlation factors for the entire organism. In like manner the placental activity is absorbed as well as those other tissues concerned with the storage of nitrogen which is a feature of this physiological condition (1, d). Further, there is the marked drop in the curve between 30 and 22 weeks which is unexplained by the limitation of active processes to the single one of foetal development. Seemingly, the mechanism is not so simple as was at first concluded and other forces certainly play a part, initially in lowering the basal rate to a subnormal level, and later conditioning a progressive return to a point superior to that of the initial downward departure.

Summary

This paper may be briefly summarized as follows:

1. The heat production of a series of 77 women throughout the last 6 lunar months of normal pregnancy is analyzed.

2. It is demonstrated that during the 3rd to 4th month of gestation there is a rapid decline in the energy requirement from a normal to a subnormal level, the latter reached in about 4 weeks.

3. From this point on, during the last 6 lunar months there is a steady increase in the basal metabolic rate amounting to 13 per cent or more in excess of that conditioned by the gross increase in body weight.

4. The conclusion that this excess is contributed primarily by the foetus has been analyzed and seemingly found not to be supported by fact. The constancy of heat output in terms of surface area when the foetal area is added to that of the mother is apparently artifactual and results from the application of a small progressive factor as a correction to a larger quantity also showing progressive incremental change but at a slower rate.

5. The excess heat production is apparently the result of a complicated and unknown mechanism, engendered by the state of pregnancy but involving other factors than those of foetal tissue growth alone.

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THE PROTEIN REQUIREMENTS OF THE ALBINO MOUSE

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THE experiments of Wheeler (11) seemed to indicate that the mouse requires a higher level of dietary protein than does the rat. Using the so-called synthetic type of food mixtures, Beard (2) claimed the growth of the mouse was stunted with diets containing less than about 23 per cent of the total calories in the form of a complete protein.[1] It may be remarked, however, that even though most of the recent nutrition studies with mice have been made with diets containing about 25 per cent casein, the protein concentration of the usual rations fed to breeding colonies has been considerably less. Dawburn (3) has reported good growth with a diet containing 15.6 per cent casein. A careful reading of the published data has revealed that both Wheeler and Beard based their conclusions on the results obtained with very few animals, and Dawburn did not include experiments with higher or lower protein concentrations for comparison with the figures obtained with the 15.6 per cent casein diet. For certain studies on the nutrition of the mouse we found it desirable to feed a diet containing minimal amounts of casein and it was important to know how far the protein could be reduced in amount before it became the limiting factor for growth. There are, naturally enough, other reasons why it would be desirable to know, more precisely than at present, the protein requirements of the smallest mammal used in nutritional investigations. It seemed, for example, that a comparison of the requirements of the mouse—once these were determined—with the known requirements of the rat might throw some light on the problem of variations due to species, particularly those that might be attributed chiefly to differences in body size.

This paper presents data on the early growth of mice fed upon synthetic diets of varying casein content. Osborne and Mendel (9) showed that cystine became the limiting factor for the growth of rats restricted to diets containing low concentrations of this protein. Lightbody and Lewis (6) and Beadles, Braman and Mitchell (1) have demonstrated that such diets

[1] In this paper, unless otherwise stated, dietary protein is expressed in terms of percentages of the total calories yielded by protein.

also produce a poor growth of hair. We have made some determinations of the glutathione concentration of muscle and liver on the assumption, first, that the iodometric titration is really a measure of this substance and, second, that the production of tissue glutathione is in part limited by the supply of cystine in the diet. Accordingly the determination of tissue glutathione would indicate the effect of the diet upon what Lightbody and Lewis have termed the "more essential tissues" in contradistinction to a "less essential epidermal structure" such as hair.

Experiments

Mice of the inbred Bagg albino strain, reared in the laboratory, were removed from their mothers when three weeks old and placed in separate, false-bottom cages. They were fed the diets, described in Table I, for four

Table I

Composition of the Diets

Diet No.	0.95	1.96	4.0	7.8	15.6	23.2	49.7
Component	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Casein	12.5	25	50	100	200	300	670
Salt Mix.	40.0	40	40	40	40	40	40
Cornstarch	359.0	353	340	315	265	215	—
Sucrose	298.5	292	280	255	205	155	—
Lard	200.0	200	200	200	200	200	200

McCollum's salt mixture (*Jour. Biol. Chem.*, 1918, **33**, 55) was used, to each 100 grams of which were added 2.81 gm. of $Al_2(SO_4)_3.K_2SO_4.24H_2O$, 0.35 gm. of NaF, 0.66 gm. of $MnSO_4.4H_2O$ and 0.21 gm. of KI.

150 mg. of dried yeast and 120 mg. of cod liver oil were also administered to each mouse daily apart from the rest of the diet.

weeks, measurements of food intakes and body weights being recorded at weekly intervals. An observation period of four weeks' duration was chosen for the following reasons. It comprises the time during which the most rapid growth following weaning takes place; differences in growth rate due to sex have not become evident, and the data for males and females may be combined; and, finally, there is not the excessive fat formation that occurs sometimes in older animals and interferes with the interpretation of increases in body weight. It is also probable that limiting the period of observation would accentuate the high requirements postulated by Wheeler and Beard. Thus, Smith and Möise (10) have found that young rats may show at first a better growth if the dietary protein level is increased to 38 per cent but, if the experiment is prolonged, rats fed upon 12 per cent protein thrive somewhat better.

The protein used in the preparation of the diets was a good grade of commercial casein that was subsequently percolated several times with 0.2 per cent acetic acid to remove salts and other soluble impurities. The resulting air-dried product contained 13.2 per cent nitrogen.

Both yeast and cod liver oil were administered separately from the rest of the diet. The dried yeast was fed at a level of 150 mg. per mouse per day, a preliminary assay having shown this amount to be more than sufficient for normal growth. The preparation contained 7.05 per cent nitrogen, an amount sufficiently high to introduce a possible source of error in the experiments. The following attempt was made to evaluate this nitrogen. A group of 15 young mice were stunted by restriction to a diet low in casein. When the weight remained practically constant the dried yeast was increased to 300 mg. per day, but no change in weight during a period of one week was noted. The yeast was thereupon restored to its former level and supplements of casein were administered to some animals, cystine to others, while three mice were reserved for controls. The latter group barely maintained weight, but appreciable growth occurred in those mice receiving daily supplements for one week of either 50 mg. of casein or 1.0 mg. of cystine. It was concluded that the effect of the nitrogenous compounds of the yeast, in the amount fed, could for the present purposes be neglected.

While the weight of basal food eaten varied somewhat for individual mice, the intakes of yeast and cod liver oil were constant. Calculating the percentage of protein by weight in terms of total food intake, including the vitamin-containing adjuvants, the diets furnished 1.20, 2.47, 4.9, 9.8, 19.7, 29.2 and 63.0 per cent casein, respectively. Expressed as percentages of the total calories eaten, these average protein values become 0.95, 1.96, 4.0, 7.8, 15.6, 23.2 and 49.7 per cent. In calculating the caloric values for casein, cornstarch, sucrose and dried yeast, the factor of 4 calories per gram has been used, and for lard and cod liver oil, 9 calories per gram. The food mixture referred to as "stock" consisted of whole ground wheat 65.8 per cent, dried whole milk 32.9 per cent, sodium chloride 0.65 per cent, and calcium carbonate 0.65 per cent.

The question may be asked whether the amount of salt mixture in the diets was sufficient, because along with the supposedly higher protein requirements it has been believed that the mouse needed a higher concentration of inorganic salts. We have found, however, in some unpublished experiments, that 4 grams of a complete salt mixture per 100 grams of dried food is more than adequate for normal body growth and normal bone production. Hence, we have used this concentration because we thought it

preferable and also because it conformed with the customary usage in nutrition studies with rats.

At the age of seven weeks the mice were killed by bleeding during ether anesthesia. The muscles of the hind legs were then dissected, weighed, and the sulfhydryl content determined by Hess' procedure (5) with one or two minor alterations, that follow. The protein in samples of tissue weighing 1 to 2 grams (representing, sometimes, several animals) was precipitated with tungstic acid, the titration with M/1200 KIO_3 was done with a micro-burette, and two drops of freshly prepared starch solution were added as an indicator just before reaching the end-point. In a number of cases analyses were also made of the liver.

Results and Conclusions

The data for males and females have been pooled because no differences attributable to sex could be detected. Animals that consumed insufficient food (less than 10 grams of the basal diet per week), due to failure to eat the yeast or for unknown reasons, have been omitted from the tabulations. These mice were chiefly from groups fed diets containing 0.95, 1.96 and 49.7 per cent protein. The data for most of the individual animals may be obtained from Figure 1, whereas the values in the tables are grouped averages. As a means of indicating the variation within each group, the standard deviations of the mean values for growth and food intake have also been determined.

Food consumption. Table II shows that the average weights of basal food eaten by the mice fed diets containing 1.96, 4.0, 7.8 and 15.6 per cent protein were remarkably constant, varying only from 63.2 to 64.8 grams

Table II

Growth and Food Consumption of the Mice

Diet Casein per cent	No. of mice	Av. Wt. beginning	Av. Wt. end	Av. Gain in Wt. in 4 weeks ± S. D.	Basal Food Intake in 4 weeks ± S. D.
		gm.	gm.	gm.	gm.
0.95	4	9.3	7.8	−1.5±0.5	52.9± 6.0
1.96	6	9.4	10.1	0.7±0.9	63.5±12.1
4.0	12	8.6	11.1	2.5±1.0	63.2±10.9
7.8	10	9.1	15.4	6.3±1.6	63.5±10.6
15.6	14	9.3	17.0	7.7±1.3	64.8± 8.9
23.2	8	8.1	15.7	7.6±1.9	58.4± 6.4
49.7	3	7.7	15.6	7.9±1.3	44.8± 3.1
Stock	9	7.2	15.9	8.6±1.9	— —

in four weeks. There seemed to be a tendency for animals receiving a very low protein diet (0.95 per cent), and those receiving a very high protein diet (49.7 per cent), to eat less food. The individual variations in both groups are large enough, however, to render the observed differences of little significance. The statistical method that we have used to calculate the significance of the mean results is that described by Fisher (4), and is

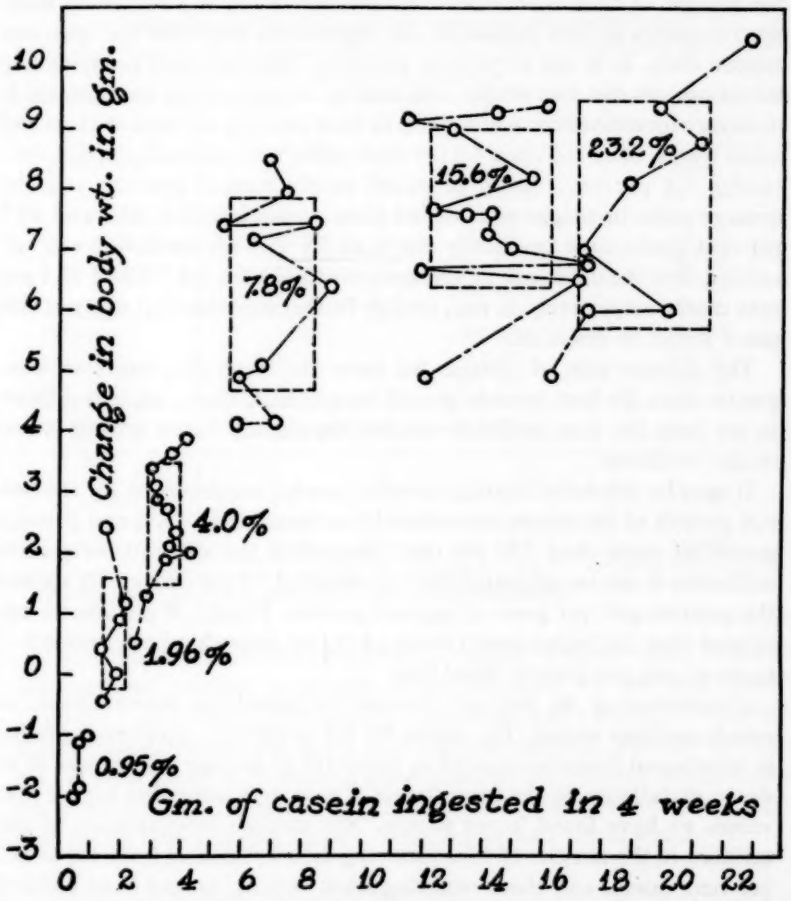

FIG. 1. The chart shows the relationship between dietary protein concentration, food intake (expressed as grams of casein eaten in 4 weeks), and the gain in weight. Each circle represents one mouse, and those animals receiving the same diet are shown connected by a dot and dash line. The dotted rectangles enclose the areas bounded by twice the standard deviation of the average growth of each group by twice the standard deviation of the average casein intake.

particularly adapted for treating small samples. To estimate Fisher's "t" the given values for the standard deviations may be converted into Σd^2 by squaring and multiplying by one less than the number of cases. This value can then be substituted in the equation developed by Fisher.

Growth. It may be noted that the level of protein in the diet containing 0.95 per cent casein is about equal to the fecal metabolic nitrogen (0.2 gm. per 100 gm. of dried food) shown by Mitchell (8) to be excreted by man, several species of farm mammals, the dog and the rat, when fed upon low residue diets. It is not surprising, therefore, that the mice receiving the lowest protein diet lost weight. Apparently, about 1.95 per cent protein is necessary for maintenance of weight, at least between the ages of three and seven weeks. Mice fed upon 4.0 per cent casein were stunted; the diet containing 7.8 per cent casein produced nearly normal growth; and the average gains in weight of mice fed diets containing 15.6, 23.2 and 49.7 per cent casein were practically identical. By Fisher's method it was calculated that the difference in average growth of mice fed 7.8 and 15.6 per cent casein, respectively, is real, though further experimental study of this point would be desirable.

The average gain of animals fed upon the stock diet was somewhat greater than the best average growth on synthetic diets, but it is difficult to say from the data available whether the slightly better growth is real or due to chance.

It may be concluded that the protein (casein) requirements for the normal growth of the mouse are certainly not more than 15.6, and perhaps somewhat more than 7.8, per cent. Regarding the efficiency of protein utilization it can be calculated that the mice fed 7.8 per cent casein showed the greatest gain per gram of ingested protein. Finally, it can also be calculated that the higher casein levels produced somewhat better growth in terms of gain per gram of basal food.

Composition of the body as indicated by glutathione determinations on muscle and liver tissues. The values for the sulfhydryl compounds present in muscle and liver are recorded in Table III as average percentages of reduced glutathione in the fresh tissues. These were somewhat higher than values we have found in rat muscle. The average concentration of glutathione in the muscles of mice receiving diets containing from 4.0 to 49.7 per cent casein, and those receiving stock rations, ranged from 0.052 to 0.064 per cent. In other words, it seemed that where growth occurred, even though it was stunted growth, normal glutathione concentrations were found in the muscles. One is tempted to conclude that if cystine be the limiting factor in the diet, muscle tissue of normal glutathione content is

produced or growth does not occur. At least, this conclusion would be in harmony with Liebig's law of the minimum. While our data on mice fed 0.95 and 1.96 per cent casein are too meager to permit any positive statements to be made, we believe further investigation would show that the dietary protein must be diminished to a bare maintenance level or less before a deficiency in muscle glutathione would occur.

TABLE III

CONCENTRATION OF REDUCED GLUTATHIONE IN MUSCLE AND LIVER

Diet No.	Av. Weight gm.	Av. Glutathione	
		Muscle per cent	Liver per cent
Experimental Animals			
0.95	7.8	.024 (1)	.130 (1)
1.95	10.1	.040 (2)	.120 (2)
4.0	11.1	.064 (2)	— (0)
7.8	15.4	.059 (2)	.154 (1)
15.6	17.0	.056 (5)	.365 (1)
23.2	15.7	.059 (4)	.326 (2)
49.7	15.6	.061 (2)	.285 (1)
Normal Controls on Stock Diet			
Age			
3 weeks	8.0	.067 (3)	.310 (3)
7 weeks	15.9	.052 (5)	.302 (5)
Adults	22.0	.043 (4)	.310 (4)

The figures in parentheses refer to the number of determinations.

The concentration of sulfhydryl compounds in the liver seems to bear a less constant relation to growth. Mice fed upon 7.8 per cent casein, although they showed good growth, had about half as much liver glutathione (one determination only) as did normal animals. Marenzi and Laclau (7) have found a decrease in glutathione in the livers of rats fed upon low protein diets. Our results may indicate that the mice fed upon 7.8 per cent casein were growing under a condition of nutritional stress.

DISCUSSION

Concerning the level of dietary protein, the present results show that the requirements of the growing mouse are very similar to those of the growing rat. Both seem to require not less than about 15 per cent of their total

calories in the form of a complete protein in order to grow at the rate which is normal for the species. However, the possibility remains that further improvements in technic might show that somewhat less than 15 per cent protein calories may be satisfactory.

The average human dietary probably contains between 10 and 20 per cent protein. Computations of the protein content of the milk of 13 species show variations from 7 to 35 per cent, averaging 20 per cent, which is also the value for cow's milk. The fact that human milk contains only 7 to 9 per cent of its total calories in the form of protein has been interpreted as indicative of the low requirements said to be characteristic of slowly growing species, plus the exemption from the demands of growing hair, and the possibly higher nutritional value of the proteins, particularly the lactalbumin.

There seems to be little justification, in fact or in theory, for the supposition that the mouse should require a higher dietary level of protein because it attains mature body weight a little more quickly than the rat. If there are any higher requirements they seem to be fulfilled by the increased food consumption per gram of body weight that in turn is due to the higher rate of metabolism. Differences, then, become apparent when the protein needs are expressed, not in terms of dietary protein level, but in grams of protein necessary to produce a unit gain in weight, or the amount of protein required per kilogram of body weight to produce either maintenance or normal growth. On such bases as these, the smaller species will require proportionately more protein. Thus, 1 gram of casein will produce 0.5 to 0.9 gram of mouse, or about 2 grams of rat, and 1 gram of protein in the form of breast milk will produce about 5 grams of baby.

Summary

Young mice were fed complete diets of the "synthetic" type in which the protein (casein) levels were, respectively, 0.95, 1.96, 4.0, 7.8, 15.6, 23.2 and 49.7 per cent of the total calories ingested. The food consumption and changes in body weight were measured between the ages of three and seven weeks. All the animals ate approximately equal quantities of food, except those fed the highest and lowest protein levels, respectively, where the intakes were less, though perhaps not significantly so. The mice fed 0.95 per cent casein lost weight, those fed 1.96 per cent maintained weight, and those fed 4.0 per cent grew at a definitely sub-normal rate. The mice fed 15.6, 23.2 and 49.7 per cent casein grew at nearly identical rates and attained almost the weight of animals fed upon a stock diet. Mice fed upon 7.8 per cent casein grew at a slightly sub-normal rate. The reduced gluta-

thione concentration in the muscles of the mice was about the same in all animals that grew, even though the growth might be stunted. The mice fed 7.8 per cent casein had about half as much glutathione in the liver as mice fed higher amounts of casein, indicating that their growth, which was also the most efficient in terms of gain in weight per gram of ingested casein, was under a condition of nutritional stress. It is concluded that, contrary to what has been believed, the protein requirements of the mouse are certainly fulfilled by diets containing 15.6 per cent casein, and probably somewhat less would also be satisfactory. These are about the same as the requirements of the rat, the differences due to metabolic rates becoming apparent when the gain in weight per gram of ingested protein is measured.

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THE HEAT PRODUCTION OF UNUSUALLY LARGE RATS DURING PROLONGED FASTING

By

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THE ability of small animals to withstand prolonged fasting is greater than has been commonly supposed. Thus, wild rats have survived without food for from 5 to 8 days (1) and laboratory-bred rats for 16 days or longer (2). In studies with rats at Yale University on the significance of various diets and diet ingredients, some unique rats were developed. The configuration of these rats did not suggest any abnormality in body composition, such as an unusually large deposition of fat. Consequently one would not expect the very large rat to have an advantage over the normal-sized rat in the ability to withstand fasting because of a greater initial reserve of bodily energy. If there were enormous deposits of fat, such as are found in animals that are about to hibernate, or in geese and pigs that have been purposely stuffed, one might expect longer resistance to fasting. An investigation was therefore planned to study the physiology of two very large rats (maximum weights 830 and 766 grams) during prolonged fasting at thermic neutrality. At frequent intervals throughout the fasts observations were made of the loss in body weight, the rectal temperature, and, as an index of the level of vital activity, of the oxygen consumption. As the metabolism of the very large rats was measured under basal conditions, i.e., on the first day of the fast, data were at hand for a comparison with the basal heat production of smaller rats. Hence, in addition, basal metabolism measurements at thermic neutrality were made on a number of other full-grown rats, some medium in size (307 to 396 grams) and others large (407 to 562 grams).

THE ANIMALS, THEIR DIETARY HISTORY AND RATES OF GROWTH

Male albino rats from the Osborne and Mendel colony were employed. With the exception of rat No. 25, all the animals had been used for a study of the influence of the rate of growth upon metabolism. As the ultimate weights attained by the rats varied with the kinds of diet and the rates of growth, these rats, when full-grown, were employed for control purposes in

the study of the *basal* metabolism of the unusually large rats. The medium-sized rats, Nos. 1 to 12, had gained weight at the rate of about 2 grams a day during the normal period of growth. These rats were fed a synthetic diet,[1] with daily supplements of yeast in amounts to secure the desired increment in weight. At the time that the metabolism measurements concerned with size were made, the medium-sized rats had subsisted upon the experimental rations, on the average, about 230 days. The large rats, Nos. 13 to 24, had gained about 4 grams a day during the period of most rapid growth. These rats were fed a synthetic diet[2] supplemented daily with dry brewers' yeast (200 to 400 mg.) and 20 grams of fresh lettuce. The large rats had received the special diets for about 250 days when the metabolism measurements under discussion were made. Some of the "slow growth" rats and some of the "rapid growth" rats received cod liver oil, but this adjunct was not universally fed.

The two unusually large rats each had a special history. Rat No. 26 gained on the average 5.0 grams per day for 55 days, between the age of 23 and 78 days. Although the average daily gain of this rat did not exceed the best growth records that have been reported for the normal rat (6.1 grams (3) and 7.3 grams (4) during the period of growth from 60 to 300 grams), a gain of 276 grams in 55 days is an exceptional weight increment. At first, the diet of rat No. 26 (23 to 331 days) was similar to that of rats Nos. 13 to 24. With this diet, growth ceased when the animal weighed 664 grams. Then, for the next 96 days, the rat received rations consisting of a "special" dog biscuit, wheat germ food[3] and fresh lettuce. Occasionally the wheat germ food was replaced by milk food.[4] During the first 63 days of this dietary regimen, the weight of the rat increased from 664 to 820 grams. During the last 33 days the weight fluctuated between 810 and 830 grams. The other very large rat, No. 25, had been fed dog biscuit and a variety of other foods, including lettuce and bananas. The growth rate of this rat was not known, nor was there information concerning the exact age of the rat. The animal was probably in the second year when the metabolism was studied.

All the rats except No. 25 were brought to the metabolism laboratory when about 30 days of age. They were housed separately in cylindrical metal cages (5). The temperature of the environment ranged between 23°

[1] Casein, 18 per cent; Osborne and Mendel salt mixture, 4 per cent; cornstarch, 54 per cent; butter fat, 9 per cent; lard, 15 per cent.

[2] Casein, 35 per cent; Osborne and Mendel salt mixture, 4 per cent; cornstarch, 37 per cent; butter fat, 9 per cent; lard, 15 per cent.

[3] Whole milk powder, 50 per cent; wheat germ, 20 per cent; lard, 30 per cent.

[4] Whole milk powder, 60 per cent; cornstarch, 12 per cent; lard, 28 per cent.

and 25°C. Rat No. 25 had not been confined in a small cage during the major portion of its life, but had wandered about in a large room during the day. Overnight it was confined in a standard rat cage. Rat No. 25 was brought to the metabolism laboratory 20 days before the long fast and 8 days before the first metabolism measurement. During this 20-day period, rat No. 25 was caged like the other rats. It lost 36 grams in weight during the period of acclimatization to the metabolism laboratory.

Rat No. 26 began its prolonged fast when it was 430 days old. It fasted from December 18 to February 13. During the fasting period it was kept in a special room at a constant temperature (26° to 28°C.). Water was available at all times, except during metabolism measurements. A raised screen of coarse wire mesh was placed in the rat cage for the first 5 days of the fast, i.e., the period during which food residues might be excreted. Thereafter the screen was not used. It was our intention to let rat No. 26 fast until death, but the importance of making certain histological studies seemed to warrant killing the animal on the 57th day of the fast, before it actually succumbed. The autopsy was performed immediately, and the findings are noted subsequently in this report. Rat No. 25 fasted from June 9 to July 17 at the prevailing environmental temperature. During the long fast it was caged like rat No. 26 and also had access to water. With rat No. 25 the plan was to refeed the animal and then initiate a second fast. The purpose of realimentation was to determine whether the rat would regain its initial weight and at what rate, and whether it could then withstand a second fast as long as the first. Hence, on the 38th day of the fast, when the animal had lost 45 per cent of its initial weight (730 grams), food[5] was again given. At this time the rat appeared well and in a normal condition, without external evidences of debilitation or disease. After the 44th day of realimentation the rat, without apparent cause, suddenly began to lose weight and died 9 days later. A post mortem examination did not reveal any anatomical abnormalities.

Technic

The oxygen consumption was measured with the multiple-chamber respiration apparatus previously described (6). Owing to the great size of the animal, a special chamber was built for rat No. 26. The rat was left inside the respiration chamber for adjustment for approximately one hour. The exact time required for the animal to absorb from the closed ventilating circuit a known volume of oxygen (about 370 cc.) was determined for 3

[5] The diet consisted of dog biscuit, milk food or wheat germ food, and lettuce.

or more successive periods, these periods varying in length from about 40 to 90 minutes. In the final tabular presentation of results the average of 3, occasionally 2, and rarely 4 successive determinations has been used. The data are reported in the following tables as the oxygen consumption per 6 hours, the heat production per kilogram of body weight, and the heat production per square meter of body surface per 24 hours. Since it is known that the respiratory quotient of the fasting rat is essentially 0.72, the heat production has been calculated from the measured oxygen consumption on the assumption that for each liter of oxygen consumed 4.702 calories are produced. In the calculation of the surface area the Rubner formula $S=9.1\times w^{2/3}$ was used.

Theoretically, strict comparisons of metabolism measurements are possible only when animals are completely immobile. That with rats is impossible, although we have found that there is a period between 10 A.M. and 4 P.M. when rats are usually quiet. The metabolism measurements, both in the basal periods 24 hours after food and during the prolonged fasts, were always made between these hours. The usual technic of recording graphically the degree of repose or activity of the rat was employed. With the large rats, the activity was frequently not recorded so that a large animal cage could be used in the regular respiration chamber. For this reason, activity records were not secured during the basal metabolism measurements of some of the large rats (407 to 562 grams), nor was the activity of rat No. 25 recorded during metabolism measurements early in the long fast. From the kymograph records we have estimated, in accordance with our usual procedure, the percentage of time during the experimental period when the animal was active. These estimates of activity are recorded in the tables in all instances where they were obtained. Although it is impracticable to compare the activity in the different experiments too closely from the quantitative standpoint, it was obvious that the rats as a whole were not extremely active inside the respiration chamber. It is difficult to evaluate to what extent such chamber activity influences the metabolism. Although our estimates of activity are given a quasi-mathematical relationship by being expressed as percentages, these estimates must be taken with considerable reserve.

For metabolism measurements under basal conditions, food was withdrawn 24 hours prior to the experiment. During this interval the fasting rats lived in a special glass cage at a temperature not far from 28°C. The metabolism was measured at 30°C., both in the basal periods 24 hours after food and during the prolonged fasts. Each rat was weighed at the close of its metabolism measurement. The rectal temperature was determined

usually within 15 minutes after the end of the experiment. Observations upon the rectal temperatures were made with a one-minute clinical thermometer. With most small animals the determination of the body temperature is difficult. There is usually a great deal of struggle on the part of the animal, and consequently a momentary increase in temperature caused by the activity. There is likewise a cooling effect produced in turning the animal over on its back and exposing its abdomen to the air. Our animals were gentle, however, and especially advantageous conditions were obtained for securing the actual body temperatures. Thus, the rats were handled by a laboratory caretaker, accustomed to holding them. Feces, when present, were first expelled from the rectum by gentle pressure. The thermometer, well lubricated, was then inserted to a depth of at least 4 cm., and read at the end of one minute. The animals seldom struggled during the entire procedure.

Duration of Fasts; Losses in Body Weight

The body-weight changes of our two very large rats during the periods of prolonged fasting are shown in Table I. Rat No. 26, which weighed 822 grams at the beginning of the fast, lived until the 57th day when it was killed and autopsied. On this day the weight had reached 376 grams, which was only 46 per cent of its initial weight. The average daily losses in weight and the per cent daily losses have been computed for both rats. The immediately preceding weight has been used each time as the baseline. On the first day of fasting rat No. 26 lost 25 grams, or 3.0 per cent of its weight. Rat No. 25 lost almost the same amount, 24 grams, but since it weighed 730 grams at the start, this corresponded to a loss of 3.3 per cent per day. The daily rate of loss remained relatively constant with rat No. 26, always a little over 1 per cent, until the 46th day of the fast. From then on, with the exception of the 50th day when the animal may have eaten feces from another rat, the percentage decrease in weight was somewhat greater.

Rat No. 25 weighed 730 grams when food was withdrawn and 398 grams on the 38th day of the fast, or 55 per cent of its initial weight. With this rat, after the first few days, the loss remained relatively constant from day to day at about 7 or 8 grams, approximating about 1.6 per cent daily of the preceding weight. When rat No. 26 had been without food for 40 days, its weight was 60 per cent of its initial weight. In other words, rat No. 26 was losing weight less rapidly than rat No. 25. Several factors may have contributed to this conservation of body tissue. In the first place, rat No. 26, although larger than rat No. 25, had a lower total metabolism (see

Table III). Secondly, rat No. 26 was inactive throughout the entire fast, whereas rat No. 25 was more restless. Cage life was relatively new for rat No. 25. This factor possibly contributed to the greater degree of restlessness of this rat. The environmental temperature was also controlled to within narrow limits for rat No. 26, whereas rat No. 25 was exposed to the prevailing environmental temperatures (summer) save when in the respiration chamber.

TABLE I

BODY WEIGHT CHANGES OF TWO VERY LARGE MALE RATS DURING PROLONGED FASTING

Rat No. 26				Rat No. 25			
Duration of fast	Weight of rat	Average daily loss	Per cent daily loss	Duration of fast	Weight of rat	Average daily loss	Per cent daily loss
days	gm.	gm.		days	gm.	gm.	
0	822	—	—	0	730	—	—
1	797	25	3.0	1	706	24	3.3
5	748	12	1.5	3	685	11	1.6
9	716	8	1.1	8	637	10	1.5
12	688	9	1.3	10	623	7	1.1
15	665	8	1.2	15	580	9	1.4
19	634	8	1.2	17	562	9	1.6
22	612	7	1.1	22	524	8	1.4
26	588	6	1.0	24	512	6	1.1
29	566	7	1.2	29	473	8	1.6
33	544	6	1.1	32	440	11	2.3
36	516	9	1.7	35	422	6	1.4
40	494	6	1.2	38	398	8	1.9
43	476	6	1.2				
46	452	8	1.7				
48	433	10	2.2				
50	*427	3	0.7				
52	410	9	2.1				
54	397	7	1.7				
56	382	8	2.0				
57	376	6	1.6				

* Two days before this weight was determined rat No. 26 was photographed. Possibly the animal ate feces from another rat during the time it was not in its own cage.

The draft upon body weight was more rapid and heavier toward the end of the fast with both animals, particularly with rat No. 26. Since fasting organisms in general tend to become richer in water, this is all the more significant. Indeed, one might expect that in these two cases the protein storage was being drawn upon, although the autopsy of rat No. 26 showed a liberal reserve of fat. This finding makes it particularly unfortunate that the nitrogen excretion was not directly determined.

Since the total life span of the laboratory-bred albino rat rarely exceeds three years, our rat that fasted 57 days was without food for about one-twentieth of its probable total life. This fasting period with the rat would correspond to 4 1/2 years without food in the average life span of man.

Resistance to fasting (autopsy findings). As the fasts progressed, it was obvious that these very large rats were capable of withstanding complete withdrawal of food far longer than anyone would have predicted. There were no evidences of debility and the animals were in no sense approaching a moribund state. The generally good condition of rat No. 26, even at the termination of the fast, was confirmed by a post mortem examination,[6] which showed that after 57 days without food considerable quantities of mesenteric and perirenal fat were still present. All the fat of the omentum was gone and the membrane had degenerated. The cause of the peculiar eye condition of rat No. 26 was diagnosed by a special study of the tissues. There was no evidence of vitamin A deficiency. The eyes were sunken into their orbits and showed a serosanguineous discharge. The Harderian glands were atrophied. With the exception of these degenerative changes, the rat was still in good condition. The lungs were normal, the heart was not enlarged, the liver was pale in color and large, but not any greater in size than has usually been observed in "rapid growth" rats. The kidneys were congested and large; the testes small and flaccid. The stomach showed hypertrophy; the glands at the base of the tongue were normal. A histological examination of the tissues of the various organs was made.[7] The tissues were compared with those of rats that had a similar dietary history but had not been subjected to a long fast. The thyroid was normal. A moderate amount of colloid was present with the usual variation. There were not any greatly enlarged acini nor areas of hyperplasia without colloid. The liver was greatly altered. The cytoplasm formed a net resembling a honeycomb with nuclei scattered through this apparent syncytium. The testes were greatly atrophied with no sign of spermatogenesis. The diameter of the seminiferous tubules was small. In the adrenal there was perhaps a slight tendency toward cytoplasmic vacuolization in the medulla. The kidney showed vacuolization of the cells of the convoluted tubules and also in the collecting ducts.

Realimentation after prolonged fasting. During the first 44 days of realimentation, rat No. 25 increased in weight from 398 to 592 grams, repre-

[6] Dr. A. M. Yudkin, of the School of Medicine of Yale University, kindly performed the autopsy.

[7] The authors are indebted to G. B. Moment of the Osborn Zoological Laboratory, Yale University, for the histological studies.

senting an average daily increment of 4.4 grams. This rate of gain during the recovery from the prolonged fast compares favorably with the optimum rate of growth in *normal young male rats*, with which daily increments of from 6.1 to 4.8 grams have been found during the period of growth from 60 to 300 grams (7). Moreover it approaches that found in *resumed* growth after long periods of suppression by inadequate diets. Mendel and Cannon (7), for example, in a recalculation of earlier results of Osborne and Mendel (8), have reported daily increments of from 7.6 to 5.9 grams in such instances.

The Basal Metabolism of Full-Grown Male Rats of Various Sizes

Our basal metabolism measurements on full-grown male rats, not only those of normal size but particularly those of unusually large size, are recorded in Table II. We have made no attempt to collect all the gaseous metabolism data on male rats published in the literature heretofore, but present our own measurements here primarily so that we may have, for comparison with the very large rats, observations on normal-sized rats from the same colony, living under precisely the same conditions. The basal metabolism of all the rats except No. 25 was measured several times during the period of growth and again when maturity was reached. Only the measurements obtained on mature rats are recorded in Table II. In many instances two measurements, 20 days apart, were made while the rats remained stationary in weight. Since the two determinations usually agreed well, only one result is presented in Table II. The metabolism of rat No. 25 was determined twice under basal conditions, once 12 days before the fast and again on the first day of the long fast. The basal metabolism of rat No. 26 was measured only once after its maximum size (830 grams) had been attained. This determination was made on the first day of the long fast.

The effect of size *per se* upon the metabolism is strikingly shown by the data in Table II. With animals of the same species it is commonly found that the smaller animal has the larger heat production per unit of weight. This is the case with the rats listed in Table II. Thus, the medium-sized rats weighing from 307 to 396 grams produced on the average 91.8 calories (range from 80.5 to 100.0 calories) per kilogram of body weight per 24 hours, whereas the larger rats weighing from 407 to 562 grams produced on the average 77.4 calories (range from 69.0 to 83.0 calories). It is usually assumed that the calculation of the heat production per unit of surface area eliminates the effect of differences in size, but even on this basis the larger rats have a definitely lower heat production than the normal-sized

rats. The larger rats showed a heat production varying from 587 to 731 calories per square meter of body surface, or on the average 664 calories. The values for the medium-sized rats varied from 615 to 774 calories, and averaged 708 calories.

The basal heat production of the two unusually large rats, Nos. 25 and

TABLE II

BASAL METABOLISM OF FULL-GROWN MALE RATS OF VARIOUS SIZES
(Measured at 30° C. and 24 hours after food.)

Rat No.	Age	Body* weight	Rectal temp.	Heat production per 24 hours		Activity
				Per kg.	Per sq. m.	
	days	gm.	°C.	cal.	cal.	%
1	232	307	37.2	100.0	740	12
2	285	308	37.9	92.5	687	—
3	204	313	37.9	97.5	726	22
4	170	323	38.4	94.5	714	11
5	239	338	37.9	80.5	615	10
6	267	338	37.6	88.5	676	18
7	302	344	38.2	91.0	700	—
8	302	364	37.8	86.0	676	3
9	273	372	36.9	88.5	699	10
10	272	378	37.8	90.5	717	11
11	277	378	37.0	96.5	766	—
12	281	396	36.8	96.0	774	1
Average	—	—	37.6	91.8	708	—
13	330	407	38.5	81.5	664	—
14	224	429	37.4	77.5	643	8
15	274	434	37.6	75.5	626	7
16	294	440	37.2	74.0	620	7
17	249	456	36.8	82.5	699	—
18	325	463	35.3	69.0	587	—
19	251	465	37.7	78.0	667	9
20	299	473	37.9	77.5	661	6
21	281	518	37.9	83.0	731	—
22	266	538	37.2	81.0	722	—
23	288	557	38.3	76.5	690	—
24	269	562	37.8	73.0	661	—
Average	—	—	37.5	77.4	664	—
25	—	723	38.0	63.0	621	—
	—	706	37.4	73.5	717	—
26	430	797	37.4	59.0	603	—

* The length from nose to anus was, on the average, 24.7 cm. for rats 1 to 12, and 26.4 cm. for rats 13 to 24. The length of rat No. 26 was 28.7 cm. The length of rat No. 25 was not determined.

26, was lower than that of the other two groups of rats. Thus, per kilogram of body weight the heat production of rat No. 26 was 59 calories or lower even than the minimum value of 69 calories for rat No. 18 in the group of large-sized rats. Similarly, rat No. 25 on this same basis had a heat production of 63 calories, which is measurably lower than the minimum value for rat No. 18. On the other hand, when the basal metabolism of rat No. 25 was measured a second time, a higher value of 73.5 calories was noted. This is substantiated by another high value on the 8th day of fasting (see Table III). The highest basal value for rat No. 25, however, is lower than the average of 77.4 calories for the group of large-sized rats in Table II, and the picture is consistent in that the larger the rat the lower is the heat production per unit of body weight. On the body-surface basis likewise the unusually large rats, in general, have a lower heat production than either the medium-sized or large-sized rats, 603 and 621 calories as compared with 708 and 664 calories. Rat No. 18, weighing 463 grams, is an exception. This rat actually had as low a heat production as 587 calories. But the data in general in Table II indicate a trend for a lower metabolism per unit of surface area, the larger the rat.[8]

Although there is a certain degree of lability in the rectal temperatures of normal rats and many observers have had difficulty in obtaining them, there is nothing in the picture of the temperature of these very large rats to indicate an abnormally low cell temperature. Indeed, they were quite within the range noted with normal rats.[9] The low basal metabolism of the larger rats, and particularly the enormously large rats, is therefore not due to a low cell temperature.

The number of basal metabolism measurements on the "giant" rat reported in the literature is small. The interest in these animals centers chiefly around the effect of growth-promoting extracts of the anterior pituitary upon the basal metabolism. Lee (9) found that with 4 rats (sex not stated), ranging in weight from 336 to 512 grams, the average heat production in 16 determinations over a period of 5 weeks was 697 calories per square meter of body surface per 24 hours, with a standard deviation of ± 21 calories. These animals had been injected with the extract. Control rats (females) were selected of the greatest size possible and ranged in weight from 200 to 270 grams. Nine determinations with these rats showed

[8] These *actually measured* values for the basal metabolism of these male rats on the surface-area basis are all appreciably lower than the average value of 800 calories *suggested* by Benedict and MacLeod (*Journal of Nutrition*, 1929, 1, 392) as the basal metabolism of the male rat at thermic neutrality.

[9] For the literature on the body temperature of the normal rat see S. V. Gudjonsson, *Jour. Physiol.*, 1932, **74**, 73.

a heat production of 805 calories, with a standard deviation of ±50 calories, which Lee states agrees well with the average of a large number of determinations on other normal rats under the same conditions (measured at 29.5° C.). "Giant" rats produced by hormone feeding may possibly be considered to be wholly unphysiological, but so far as the evidence is concerned it shows that the "giant" rats produced with growth-promoting extracts had a lower heat production than the smaller rats.

Gaseous Metabolism and Heat Production of Unusually Large Rats During Prolonged Fasting

The gaseous metabolism of the two very large rats, Nos. 25 and 26, was determined during their prolonged fasts every 3 or 4 days. Owing to the seemingly important changes in the type of metabolism of rat No. 26, this animal was studied every other day toward the end of the fast. The results of these metabolism observations, together with the rectal temperatures noted on the same days, are given in Table III.

In general one can say that there is no pronounced difference between the rectal temperatures of these very large rats and the body temperatures commonly reported for the rat of normal size. With neither animal was there any significant change in rectal temperature due to fasting. It would appear, therefore, that at an environmental temperature of 27°, and particularly after a stay of several hours at 30°, the rectal temperatures of these very large fasting rats, even after they had lost more than half their initial weight, were essentially normal.

With rat No. 26 from the 29th day until the end of the fast on the 56th day, the total oxygen consumption per 6 hours was fairly constant, although the body weight was decreasing rapidly during this time. Indeed, after the first 20 days, during which there was a rapid decrease in oxygen consumption, the metabolism of the organism as a whole can be considered to have remained essentially unchanged. At the end of the fast, the total oxygen consumption was only about 8 per cent less than it was on the 22nd and the 26th days. Still, the total oxygen consumption had been somewhat lower between the 29th and the 46th days of the fast, the average value being about 19 per cent less than the total oxygen consumption on the 22nd and the 26th days. Rat No. 25 did not fast as long, but here again beginning at about the 22nd day the metabolism of the organism as a whole remained constant until the 38th day, in spite of the fact that there was a considerable loss in body weight during this time. With rat No. 25 the level of the oxygen consumption at the beginning of the fast was 2751 cc., although this was definitely higher than that measured

TABLE III

METABOLISM OF UNUSUALLY LARGE MALE RATS DURING PROLONGED FASTING
(Living at 27° C.; measured at 30° C.)

Rat No. and duration of fast	Body weight	Rectal temp.	Oxygen consumed per 6 hours	Heat production per 24 hours		Activity
				Per kg.	Per sq. m.	
days	gm.	°C.	cc.	cal.	cal.	%
Rat No. 26*						
1	797	37.4	2505	59.1	603	—
5	748	37.9	2189	55.1	549	10
9	716	37.3	2002	52.7	517	9
12	688	37.3	1751	47.8	464	8
15	665	38.0	1713	48.4	465	10
19	634	37.8	1722	51.1	482	11
22	612	37.8	1552	47.7	445	11
26	588	37.7	1624	51.9	478	12
29	566	37.0	1248	41.5	377	5
33	544	37.8	1618	55.9	502	7
36	516	37.4	1268	46.3	408	4
40	494	37.3	1466	55.9	485	8
43	476	36.0	1124	44.3	381	4
46	452	36.8	1187	49.3	417	6
48	433	37.3	1400	60.7	506	9
50	427	37.4	1432	63.0	522	6
52	410	37.6	1470	67.3	551	9
54	397	37.1	1441	68.3	551	6
56	382	37.4	1434	70.7	563	11
Rat No. 25*						
1	706	37.4	2751	73.2	717	—
3	685	38.1	2363	64.8	628	—
8	637	37.8	2530	74.7	706	—
10	623	37.6	2252	68.1	638	—
15	580	37.4	1974	64.0	586	—
17	562	37.8	1965	65.8	596	—
22	524	37.9	1655	59.4	527	—
24	512	38.1	1840	67.6	595	13
29	473	37.8	1635	65.1	557	7
32	440	37.9	1709	73.0	611	10
35	422	37.3	1612	71.8	592	10
38	398	37.8	1653	78.1	632	17

* Rat No. 26 was 430 days old at the start of the fast; the age of rat No. 25 was not known but it was probably in its second year.

a few days before under the usual basal conditions. From the 22nd to the 38th day of fasting the oxygen consumption averaged 1684 cc., or 61 per cent of that at the start. At the beginning of the fast the oxygen consump-

tion of rat No. 26 was 2505 cc. per 6 hours. From the 26th day on it averaged 1393 cc., or 56 per cent of that at the start of the fast.

The results for the total oxygen consumption of these unusually large fasting rats are in striking contrast with the values for fasting female rats studied earlier (10). Thus, female rats fasting 16, 15, and 24 days, respectively, had an oxygen consumption (at 28°) in all cases at the end of the fast less than 50 per cent of the initial value. With the very large rats, Nos. 25 and 26, the metabolism at the end of the fast was 61 and 56 per cent of the initial level. The female rats weighed on the average 210 grams at the start of the fasts. The very large rats weighed three and one-half times as much. Since the 3 female rats died when the oxygen consumption had fallen to approximately half of what it was at the start of the fast, one would not have expected the very large rats to die for some time after 56 and 38 days. It is regretted that rat No. 26 was killed and that the realimentation experiment was inadvisably carried out with rat No. 25. It is obvious from these comparisons alone that these unusually large rats were amply provided with reserves to withstand a very long fast. At the end of 40 to 50 days of fasting their organisms still had a high metabolism. At the end of their fasts the female rats weighed on the average 114 grams, and the oxygen consumption averaged 514 cc. per 6 hours. The average weight of the two very large rats at the end of their fasts was 390 grams, or over 3 times that of the female rats, and the average oxygen consumption was 3 times greater. On this basis one might expect that the very large rats were approaching death. From the autopsy records, however, it is clear that they had still an abundant reserve.

Since the very large rats were losing weight continually during the fast and the oxygen consumption remained at a fairly constant level, it is not surprising to find that the heat production per kilogram of body weight definitely increased toward the end of the fast. In this respect the very large rats differ again from the female rats, the heat production of which per kilogram of body weight remained essentially constant after the first one or two days of fasting. With rat No. 26 the heat production per unit of weight decreased markedly until the 12th day. It reached a minimum on the 29th day and then returned to a value even higher than it was at the beginning of the fast. With rat No. 25 the values are more variable, probably due to the activity, which was not recorded in the first 7 experiments. As this rat had not been confined in a small cage for a very long time, it was probably less content in the respiration chamber than was rat No. 26. The minimum value with rat No. 25 was found on the 22nd day and the maximum value on the 38th day. The longest fast with the female

rats was only 24 days, but the uniformity in their heat production per kilogram of body weight after the first few days of fasting is in striking contrast to the increases and the variability noted with the very large rats.

Rubner has pointed out that the heat production per unit of surface area does not follow the "surface-area law" in fasting animals, but that there is a pronounced decrease in the heat production on this basis during fasting. This decrease was shown to some extent by both of our very large rats, but not throughout the entire fast. Thus, the heat production per square meter of body surface per 24 hours, in the case of rat No. 26, *decreased* as the fast progressed until the 29th day, when it reached a minimum. Toward the end of the fast, however, it *increased* to a level as high as it was on the 5th day. With rat No. 25 there was a decrease in the heat production per square meter of surface area during the first 15 days of the fast. The day-to-day variability in the heat production of this rat, however, makes it difficult to estimate the real change in metabolism. The heat production per unit of surface area observed on the first day of prolonged fasting was unusually high, 717 calories. The basal metabolism of this same rat measured 12 days before the long fast (see Table II) was only 621 calories. Again on the 8th day of the prolonged fast a high heat value was observed, 706 calories, as compared with 628 calories on the 3d day and 638 calories on the 10th day. From the 15th to the 29th day the heat production was slightly lower, with a variability up and down from a minimum of 527 calories on the 22nd day to a maximum of 595 calories on the 24th day. From the 32nd to the 38th day of the fast the heat production was somewhat higher, 592 to 632 calories per square meter of surface area. Here toward the end of the fast there is no indication of a consistent decrease in the heat production per unit of surface area. In contrast to these results, the heat production of female rats was found to decrease fairly regularly throughout the course of fasting (10).

Comparison of our two very large rats shows that in general rat No. 25 had a higher metabolism than rat No. 26. If the two rats are compared only during the first 38 days of fasting, one sees that the average heat production of rat No. 26 was measurably under 500 calories per square meter of body surface, whereas that of rat No. 25 was almost 600 calories. It is clear that we were dealing here with definite individuality in the two animals.

The body surface of the very large rats was computed from each individual body weight obtained during the progress of the fast. This immediately brings up the question as to what is the surface area of a fasting rat. The surface area of a normal rat is usually considered to be its skin area. The skin area is determined with difficulty, owing to the danger of

stretching the skin, but many writers have assumed that with normal rats the surface area and the skin area are readily computed from the two-thirds power of the body weight times the constant 9.1. The skin area as the fast progresses must automatically become much larger than the surface area of the body. Indeed, if the heat production of these fasting rats were calculated per unit of skin area, the results might be considerably lower than those recorded in Table III, possibly as low as 300 calories per square meter of skin area. It is inconceivable, however, that the skin area of a fasting rat weighing half as much as it did at the start of the fast would even approximate half the initial skin area. It is furthermore inconceivable that the computation of the skin area as such can be made by applying the constant 9.1 to the two-thirds power of the body weight of the emaciated rat. At the end of the fast the skin of rat No. 26 had not shrunk to correspond to its lower weight. The skin was wrinkled and hung in folds and was probably much the same in area as at the start of the fast. On the other hand, if we are dealing with surface area in contradistinction to skin area, it is not impossible to assume that at least an approximation of the surface area may be obtained by the formula $S = 9.1 \times w^{2/3}$. For want of a better method of expression, this formula has been used in our calculations of the heat production per square meter of surface area during the fasting periods. Precisely the same procedure was carried out in the earlier studies with fasting female rats.

It is clear from inspection of the values for the total oxygen consumption and the heat production per unit of weight and of surface area that the metabolism of our two very large rats during the latter parts of their fasts was definitely increased. Activity cannot account for the increase. Since the rectal temperatures were normal, there could not have been an increased cell temperature to account for it. As was pointed out in the analysis of the losses in body weight during the fasts, an increased protein disintegration must be thought of, but unfortunately the nitrogen in the excreta was not determined. Comparison of the very large fasting male rats with the fasting female rats of normal size makes it seem probable that the very large rat is an altogether different organism from the normal-sized rat and that its heat loss is regulated by different laws. It serves admirably to illustrate how deficient is our knowledge of the physiology of fasting, in spite of the extensive research with both man and animals carried out in the past few decades. The fact that it is possible for a rat to grow to such a huge size and show no abnormality in configuration or general condition, and yet have a metabolism so different from that of

rats of normal size, suggests lines of study that must be carried out in the near future.

Summary

Two unusually large male rats (maximum weights 830 and 766 grams) fasted 57 and 38 days, respectively, when one was killed for autopsy and the other was refed. In neither instance did the animal appear moribund. At the end of the fasts the body weights were 46 and 55 per cent of the initial weights (822 and 730 grams). With both rats the daily loss in weight remained relatively constant, after an initial pronounced decrease, until toward the end of the fast when the drafts upon body weight were heavier. The autopsy of the rat that fasted 57 days showed it still had a liberal supply of fat.

In a comparison of the *basal* metabolism of the very large rats with smaller rats, it was found that the metabolism was lower per unit of weight and per unit of surface area, the larger the rat. Male rats weighing from 307 to 396 grams produced on the average 92 calories per kilogram of body weight and 708 calories per square meter of body surface per 24 hours. Male rats weighing from 407 to 562 grams produced 77 calories and 664 calories, respectively. The two unusually large rats produced 59 and 63 calories per unit of weight and 603 and 621 calories per unit of surface area. The rectal temperatures of the very large rats (37.4°) were similar to the body temperatures of the medium-sized rats (37.6°) and the large-sized rats (37.5°C.).

During the prolonged fasts the total oxygen consumption decreased until about the 20th day of the fasts, but thereafter remained relatively constant, although the rats were losing weight rapidly. The heat production per kilogram of body weight and per square meter of body surface decreased markedly during the first few days, but toward the end of the fasts definitely increased. This is in striking contrast to the reaction shown by fasting female rats, whose heat production per unit of weight was essentially constant after the first one or two days, and per unit of surface area decreased as the fast progressed. With the two very large rats there was no increase in cell temperature or increase in activity to account for the increased metabolism toward the end of the fasts. Indeed, the rectal temperatures during the prolonged fasts were not different from those of rats under normal conditions. These findings suggest that there was an increased protein disintegration toward the end of the fasts, although the nitrogen in the excreta was not determined.

With one of the very large rats minimum values of 377 and 381 calories

per square meter of body surface per 24 hours were recorded on the 29th and the 43d days of fasting. This is probably the lowest heat production that has been noted with warm-blooded animals having a rectal temperature of essentially 37°C.

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CALCIUM AND PHOSPHORUS OF SALIVA IN RELATION TO DENTAL CARIES*

By

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THERE have been numerous attempts to show a relation between dental caries and the amount of calcium in the saliva. Fewer studies have considered the phosphorus content as well. Some of those who believe that the preservation of the teeth is somewhat dependent on the chemical composition of the surrounding fluid, associate the absence of decay with a relatively high salivary calcium. Bunting and Rickert (1), who determined the calcium by weighing the ignited oxide, found that when the calcium was high the enamel was dense and opalescent, while when it was low the teeth were white or blue white and had the appearance of permeability or roughness. Later work from the same laboratory (2) confirmed these findings and stated that high salivary calcium was associated with immunity to caries. In partial confirmation of these results, Horton, Marrack, and Price (3), after examining salivas of several hundred children with varying numbers of carious teeth, report that as the number of carious teeth increases, the calcium of the saliva decreases. However, they report higher salivary calcium in the early stages of caries development, which leads them to conclude that the change in calcium content is not the primary change.

On the other hand, some workers, such as Spencer-Payne (4), Clark and Levine (5), and Leonard (6), have been unable to find such relations. Clark and Levine (5), in a study of various inorganic constituents of saliva emphasize particularly that the calcium content of individual salivas varies from time to time, so that it is difficult to detect any relation which may exist between salivary calcium and the condition of the teeth. This view was confirmed by Leonard (6). More recently, Karshan, Krasnow, and Krejci (7) have made a study of salivas from a group of dental students and have found slightly higher calcium for the immunes, but "the difference is not so great as some others have reported."

In the few studies concerned with the phosphorus of the saliva there has

* This investigation was made possible by a grant from the Children's Fund of Michigan.

been little agreement. Hawkins (8) found it lower for caries free individuals than for those who were susceptible to caries, while Karshan, Krasnow and Krejci (7) found it higher. Entin and Geikin (9) and Horton, Marrack and Price (3) could find no correlation between phosphorus of saliva and caries.

We have studied the salivas of school children from seven to sixteen years of age. This was done to secure more data on the calcium and phosphorus of the saliva in relation to caries and at the same time to determine whether there would be changes in tooth condition following slight dietary improvement and whether such changes would be reflected in the salivary calcium and phosphorus. The children studied were divided into five groups:

Group I included twenty-five children with active caries. The home diet was supplemented each school day by one quart of milk, two ounces of tomato juice and eight drops of viosterol.[1] The milk was given in three portions, one half pint at the opening and at the close of school, and a pint at the noon lunch hour. The tomato juice and viosterol were mixed and given with the noon meal.

Group II included twenty-five children with active caries. These received in addition to the home diet one quart of milk and two ounces of tomato juice, but no viosterol.

Group III was a control group of thirty children with active caries and the home diet was not supplemented.

Group IV was a control group of ten children with no active caries and in most cases no fillings. The members of this group received the same supplements that were given to those in Group I.

Group V included twelve children with no active caries and in most cases no fillings. This also was a control group and no supplement was made to the home diet.

Dental examinations[2] were made four times, at the beginning and at the end of, and twice during the period of the investigation. Before any of the children received supplementary feeding there were at least three analyses of saliva for each child (Table I). Groups I, II and IV received the supplements indicated beginning Jan. 10, 1930. These were discontinued June 1, resumed with the opening of school Sept. 15 and discontinued completely

[1] The viosterol was furnished through the courtesy of the Abbott Laboratories. The product first used was that known as Viosterol in Oil 100 D. Later Viosterol in Oil 250 D was used when this change was authorized by the Council on Pharmacy and Chemistry of the American Medical Association.

[2] The authors acknowledge with pleasure their indebtedness to Dr. Dorothy G. Hard and Dr. Philip Jay for assistance with the dental examinations.

TABLE I

SUMMARY OF CALCIUM AND PHOSPHORUS CHANGES

All results are expressed in mg. per 100 cc. of saliva.

Group	Number of subjects	Number of samples		Calcium				Phosphorus			
				Range		Average		Range		Average	
		1*	2**	1	2	1	2	1	2	1	2
				mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
Ia	11	35	87	2.8–7.2	2.6–7.2	4.8	5.0	10.4–15.6	10.3–16.0	12.9	13.2
b	8	25	65	3.4–6.8	2.6–7.0	5.0	5.3	9.4–15.3	9.8–15.9	12.5	12.7
IIa	12	35	95	2.5–7.3	2.2–8.0	4.6	5.0	9.8–15.9	9.4–15.7	12.0	12.3
b	5	16	39	2.8–6.6	3.4–7.3	4.7	5.1	10.0–15.2	9.1–15.4	13.4	13.2
IIIa	11	34	90	3.4–7.4	3.5–7.5	5.3	5.8	9.7–17.0	10.4–16.8	13.1	13.4
b	12	36	98	2.6–6.4	2.8–7.4	4.9	5.2	9.8–16.3	10.1–16.8	12.8	13.1
IVa	4	12	35	3.2–6.8	2.8–6.4	4.8	5.1	9.6–16.1	9.6–15.6	13.3	13.7
b	1	3	8	4.4–6.2	4.5–7.3	5.5	5.8	10.6–11.1	10.1–13.0	11.0	11.7
Va	9	28	75	4.0–7.3	3.6–7.6	5.2	5.6	9.7–16.5	9.8–16.6	12.6	12.7
b	2	6	15	4.2–7.3	4.4–7.5	5.4	6.1	11.7–15.3	10.6–15.4	13.2	13.3

* Period 1 represents three analyses for each child.

** Period 2 represents eight analyses for each child, made during the months when dietary supplements were given.

April 1, 1931. During this time salivary analyses were made for each child once every six weeks, with the exception of summer. This gave at least eight determinations for each individual during the period.

The saliva used was paraffin activated. The samples were obtained at ten a.m. in all cases. The saliva was transferred through a short-stem glass funnel into a small flask. Collecting was continued for 15 to 30 minutes, depending on the rate of flow. The samples were analyzed for total solids, ash, calcium and phosphorus. Two ten cc. portions were transferred to 40 cc. platinum dishes and dried in an electric oven over night to determine total solids. The dried material was ashed in an electric muffle just below dull red heat and the residue was weighed. The ash was dissolved by digesting it with 1 cc. portions of hot 1:3 hydrochloric acid and the solution was transferred to a 25 cc. volumetric flask. Five cc. portions of this acid ash solution were used for the determination of calcium by the Clark and Collip (10) modification of the Kramer and Tisdall (11) method, and 0.5 to 1 cc. portions for the determination of phosphorus by the method of Fiske and Subbarrow (12).

Discussion

In summarizing the data obtained, each of the five groups has been considered in two periods. The first covers the analyses made during October, November and December 1929, before any of the children had received dietary supplements. The second division includes the analyses from January 1930 through March 1931 when an attempt was made to improve the diet for selected groups.

On the basis of the dental examination made at the conclusion of the work, each of the five groups was divided into two sub-groups, *a* and *b*. Sub-group *a* in each case includes those individuals who developed no new cavities during the time under observation, while in sub-group *b* are placed those showing new lesions or extensions of old ones. Table I summarizes data for all individuals for period 1 and period 2.

For period 1 there was no distinct difference either in range or average for the calcium or phosphorus of the saliva of children with active caries as compared with those who were caries free. Furthermore, we found rather wide variations in the calcium and phosphorus content of samples from the same individual at different times. This is shown in Table II which gives calcium and phosphorus content of salivas of six caries-susceptible and six caries-free children for the three determinations during this preliminary period. For the second and longer period, when selected individuals received supplements to the home diet, Table I shows that in Group I, re-

ceiving milk, tomato juice and viosterol, 58 per cent of those who were under observation for the whole time showed an arrest in caries activity. For Group II, in which milk and tomato juice were the only supplements, 70 per cent showed improved clinical condition, while for Group III, with

TABLE II

TYPICAL CALCIUM AND PHOSPHORUS VALUES FOR SALIVA OF CARIES-SUSCEPTIBLE AND CARIES-FREE CHILDREN

All results are expressed in mg. per 100 cc. of saliva.

Caries-Susceptible		Caries-Free	
Calcium	Phosphorus	Calcium	Phosphorus
mg.	mg.	mg.	mg.
L.A. 3.2	10.4	D.H. 5.5	14.0
4.4	9.8	4.9	12.5
5.6	10.7	6.8	13.2
M.B. 2.9	10.4	C.L. 5.0	15.1
5.3	11.6	4.4	13.0
5.1	8.5	5.2	13.8
L.H. 4.1	14.8	F.S. 4.2	12.3
6.1	13.3	2.2	11.7
5.1	14.7	3.2	13.4
A.K. 2.2	10.4	L.S. 4.6	9.7
4.3	11.5	6.8	10.0
2.1	10.3	4.7	10.5
R.S. 5.1	10.5	M.W. 5.0	10.7
7.3	10.9	4.5	9.8
7.7	12.0	4.3	10.0
R.K. 4.9	13.4	C.Y. 4.7	13.1
2.8	15.0	5.4	13.3
3.5	15.7	5.3	14.1

no supplement, 48 per cent developed no new caries. Eighty-one per cent of those in Group IV and Group V were caries free. However, the number of subjects in these two groups was so small that adequate comparisons cannot be made. Thus, there was a somewhat lower incidence of caries corresponding with dietary supplement, but the differences were not marked.

The calcium and phosphorus figures show even less change. While most of the values are somewhat higher in period 2 there is no correlation between these changes and either the diet or the arrest of caries. Moreover,

the averages of all values for the second period agree very closely with the average figures which have been obtained in this laboratory for all children, regardless of diet. A total of 902 determinations on children with active caries has given an average calcium of 5.1 and phosphorus of 12.8 mg. per 100 cc., while 275 determinations on children who were free from caries have averaged 5.4 calcium and 13.3 mg. phosphorus.[3]

While we could detect no constancy of calcium or phosphorus content of saliva and no consistent variation with changes in tooth condition or with the diet supplements used, it has become evident that the volume of saliva secreted in a given time should be considered. The average rate of flow for these children was 1 cc. per minute. There were, however, many individuals who constantly produced much more than this, and others whose rate was much lower. If we consider two extremes in rate, 0.5 and 2 cc. per minute, we find average figures as shown in Table III. The lower values for

TABLE III

VARIATION OF CALCIUM AND PHOSPHORUS OF SALIVA WITH RATE OF SECRETION

Volume	Number of samples	Calcium	Phosphorus	Calcium	Phosphorus	Total Solids	Ash
cc./min.		mg./100 cc.		mg./min.		gm./100 cc.	
0.5	65	6.0	15.0	0.030	0.075	0.650	0.182
2.0	97	4.8	11.9	0.096	0.238	0.541	0.156

calcium and phosphorus with the larger volume are not due entirely to dilution, for the figures are not proportional to the volumes. If we calculate the total amount of salivary calcium and phosphorus per minute for each group we find that those with the more rapid flow secrete about three times as much as the slower. When the calcium and phosphorus are expressed in mg. per 100 cc. of saliva much higher values are reported for those individuals with the slower rate of secretion. If the saliva is a possible factor in the determination of immunity of susceptibility to caries, it seems as if one should consider not only the relative amounts of the constituents as expressed in the usual way, but should take into account as well the total amount of material available at a given time. At present we can make no satisfactory correlation between rate of flow and tooth preservation, although there appears to be a slight tendency for the caries-free individuals to secrete larger volumes than is the case of those with active caries.

[3] These figures include some unpublished data.

SUMMARY

1. In a group of children from seven to sixteen years of age there was no relation between calcium and phosphorus content of the saliva and the occurrence of dental caries.

2. When the diet was supplemented by the daily addition of one quart of milk and two ounces of tomato juice, with or without viosterol, there was a slight tendency toward a decrease in the incidence of dental caries. This improvement in tooth condition was not accompanied by any consistent change in the salivary calcium and phosphorus.

3. Evidence has been presented that the volume of saliva secreted in a unit time should be considered in interpreting salivary analyses.

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INDEX

A

ACTION of radio-active substances on vitamins (Hogan *et al.*) 413
Adams, W. Lloyd (see Bing, Franklin C.) 571
Addition of raw beef or meat scrap to a wheat-milk diet (Russell) 347
Adolph, Wm. H., and Shen-chao Chen, The utilization of calcium in soy bean diets 379
Alfalfa or barley, comparative study of rabbits maintained on (Bischoff *et al.*) 403
Amino acids, as supplements to the soluble fraction of meat meal and corn (Curtis *et al.*) 510
Analysis of the curve of heat production in relation to the plane of nutrition (Forbes and Kriss) 183
Anderson, C. B. (see Levine, Harold) 295
Anemia, nutritional, value of some vegetables in (Levine *et al.*) 295
Antimony trichloride color reaction for vitamin A (Norris and Church) 495
Areas, foetal (Rowe and Boyd) 557

B

BACILLUS Acidophilus, failure to produce dental caries in the white rat, with (Lilly) 175
Barley or alfalfa, comparative study of rabbits maintained on (Bischoff *et al.*) 403
Barnes, B. O. (see Quigley, J. P.) 77
Bassett, Samuel H., C. A. Elden and W. S. McCann, Mineral exchanges of man. II. Effect of excess potassium and of calcium on two normal men and on an oedematous nephritic 1
Benedict, Francis G., Kathryn Horst and Lafayette B. Mendel, The heat production of unusually large rats during prolonged fasting 581
Bethke, R. M. (see Krauss, W. E.) 467
Bing, Franklin C., W. Lloyd Adams and Russel O. Bowman, The protein requirements of the albino mouse 571
Biological value of protein, method of determining in the study of avian nutrition (St. John *et al.*) 267
Bischoff, Fritz, Editorial Review, The influence of diet on renal and blood vessel changes 431
—, W. D. Sansum, M. Louisa Long and Richard D. Evans, A comparative study of rabbits maintained on barley or alfalfa 403
Blood, of dairy cattle, hemoglobin content of (Brooks and Hughes) 35
— serum, concentration of calcium and inorganic phosphorus in (Kramer and Howland) 39
— sugar, heat production and nitrogen elimination of subjects exposed to cold (Swift) 232
— — values, variations in, of normal and vagotomized dogs following glucose administration (Quigley *et al.*) 77
— vessel changes, influence of diet on (Bischoff) 431
Body weight and dietary relations of rats (Limson and Jackson) 167
Bowman, Russell O. (see Bing, Franklin C.) 571
Boyd, William Clouser (see Rowe, Allen Winter) 551
Braman, Winfred W. (see Forbes, E. B.) .. 387
Breckenridge, Gerald F. (see Hogan, Albert G.) 413
Brooks, H. J., and J. S. Hughes, The hemoglobin content of the blood of dairy cattle 35
Bunting, Russell W. (see Hubbell, Rebecca B.) 599
Butterfat, critical amount required to produce healing of rickets in rats (Krauss *et al.*) 471
—, effect of different samples of on calcification (Krauss *et al.*) 473, 474

C

CALCIUM and phosphorus of saliva in relation to dental caries (Hubbell and Bunting) 599
— chloride feeding, normal and nephritic subjects (Bassett *et al.*) 14
—, concentration of in blood serum of rats (Kramer and Howland) 39

Calcium, effect of excess of, on normal men and on an oedematous nephritic (Bassett *et al.*) 1
—, magnesium and phosphorus balances in normal and nephritic subjects (Bassett *et al.*) 12
— of navy bean, utilization of (Pittman) .. 277
— retention on a diet containing American cheddar cheese (Mallon *et al.*) 121
— salts, absorption and excretion of in rats (Kramer and Howland) 56
—, utilization of in soy bean diets (Adolph and Chen) 379
Carver, J. S. (see St. John, J. L.) 267
Caseins, comparative growth-promoting properties of (Gunderson and Steenbock) 205
Cattle, heat production of, in a respiration calorimeter as related to the rate of ventilation and to the moisture content of the air (Forbes *et al.*) 387
Changes in the weights of various organs and systems of young rats maintained on a low-protein diet (Limson and Jackson) 163
Cheese, American cheddar, calcium retention on diet containing (Mallon *et al.*) 121
Chen, Shen-chao, (see Adolph, Wm. H.) .. 379
Chicks, calcification in, effect of butterfat on, (Kraus *et al.*) 474
Child, pre-school, iron requirement of (Leichsenring and Flor) 141
Church, Anna E. (see Norris, Earl R.) 495
Comparative study of rabbits maintained on barley or alfalfa (Bischoff *et al.*) ... 403
Coons, Callie Mae, and Anna T. Schiefelbusch, The diets of college women in relation to their basal metabolism ... 459
Copper and iron content of vegetables (Levine *et al.*) 300
Cow(s), dairy, economy of conversion of food energy into milk energy by (Forbes and Voris) 395
—, effect of feeding irradiated ergosterol to on the vitamin D content of milk (Krauss *et al.*) 467
—, heat production of, related to moisture of air and water vaporized (Forbes *et al.*) 390
Culp, F. B. (see Levine, Harold) 295
Curtis, P. B., S. M. Hauge and H. R. Kraybill, The nutritive value of certain animal protein concentrates 503
Cystine and protein in the diet of the rat, relation of the vitamin B complex to renal enlargement caused by (Longwell *et al.*) 539
— supplement to navy bean diet (Pittman) 277

D

Darby, Clara R. (see Mallon, Marguerite G.) 121
Dairy cattle, hemoglobin content of blood of (Brooks and Hughes) 35
— manufacturing processes, effect of upon the nutritive value of milk (Nevens and Shaw) 485
Dental caries, calcium and phosphorus of saliva in relation to (Hubbell and Bunting) 599
——, failure to produce in rat with high carbohydrate diet and Bacillus Acidophilus or with vitamin D deficiency (Lilly) 175
Diet(s), high carbohydrate, failure to produce dental caries in the white rat with (Lilly) 175
—, influence of on renal and blood vessel changes (Bischoff) 431
—, low-protein, changes in weights of organs and systems of young rats maintained on (Limson and Jackson) 163
— of college women in relation to their basal metabolism (Coons and Schiefelbusch) 459
—, of the rat, relation of the vitamin B complex to renal enlargement caused by cystine and protein in, (Longwell *et al.*) 539
—, rachitogenic, effect of, on thyroid gland of albino rat (Thompson) 359
—, soy bean, utilization of calcium in (Adolph and Chen) 379
—, wheat-milk, addition of raw beef or meat scrap to (Russell) 347
Dietary of the human mother with respect to the nutrients secreted into breast milk (Shukers *et al.*) 127
— requirements for fertility and lactation. XXIV. Further studies on the specific

effect of vitamin B on lactation (Sure and Smith) 147
Digestibility of fresh whole milk and of evaporated milk (Nevens and Shaw). 485

E

ECONOMY of conversion of food energy into milk energy by the dairy cow (Forbes and Voris) 395
Editorial Review, Egg-yolk proteins (Jukes and Kay) 81
— —, The influence of diet on renal and blood vessel changes (Bischoff) 431
Effect(s) of a high intake of manganese on the growth of rats (Skinner) 451
— of dairy manufacturing processes upon the nutritive value of milk. I. The apparent digestibility of fresh whole milk and of evaporated milk, (Nevens and Shaw) 485
— of feeding irradiated ergosterol to cows on the vitamin D content of milk (Krauss *et al.*) 467
— of figs and small amounts of raisins on urinary acidity (Saywell) 519
— of grapes and grape products on urinary acidity (Saywell) 103
— of low environmental temperature upon metabolism. I. Technic and respiratory quotient (Swift) 213
II. The influence of shivering, subcutaneous fat and skin temperature on heat production (Swift) 227
—of rachitogenic diets on the thyroid gland of the albino rat (Thompson) .. 359
Egg, hen's, percentage composition of the proteins of the yolk (Jukes and Kay) 83
— yolk, effect of diet on composition of (Jukes and Kay) 97
— —, non-avian, percentage composition of proteins (Jukes and Kay) 90
Elden, C. A. (See Bassett, Samuel H.) 1
Ergosterol, irradiated, effect of feeding to cows on the vitamin D content of milk (Krauss *et al.*) 467
—, —, treatment of rachitic infants with milk produced by cows fed (Gerstenberger and Horesh) 479
Estrous cycle and gestation, some effects of restricted protein intake on, in the rat (Guilbert and Goss) 251
Evans, Richard D. (see Bischoff, Fritz) ... 403

F

FACTORS which determine the concentration of calcium and of inorganic phosphorus in the blood serum of rats (Kramer and Howland) 39
Failures to produce experimental dental caries in the white rat with high carbohydrate diet and Bacillus Acidophilus or with vitamin D deficiency (Lilly) 175
Fasting, prolonged, heat production of unusually large rats during (Benedict *et al.*) 581
Fat, subcutaneous, measurements of (Swift) 236
—, —, shivering and skin temperature, influence of on heat production (Swift)
Feed, partition of energy of at different planes of nutrition (Forbes and Kriss) 186
Fertility, dietary requirements for (Sure and Smith) 147
Figs, effect of on urinary acidity (Saywell) 519
Flor, Iva Hansen (See Leichsenring, Jane M.) 141
Foetal areas (Rowe and Boyd) 557
— influence on basal metabolic rate (Rowe and Boyd) 551
Food consumption of college women (Coons and Schiefelbusch) 460
— energy, economy of conversion of, into milk energy by the dairy cow (Forbes and Voris) 395
Forbes, E. B., and Leroy Voris, The economy of conversion of food energy into milk energy by the dairy cow 395
—, and Max Kriss, The analysis of the curve of heat production in relation to the plane of nutrition 183
—, Winfred W. Braman, and Max Kriss The heat production of cattle in a respiration calorimeter as related to the rate of ventilation and to the moisture content of the air 387

G

GERSTENBERGER, Henry J., and Arthur J. Horesh, The treatment of rachitic infants with milk produced by cows fed irradiated ergosterol 479

Gestation and estrous cycle, some effects of restricted protein intake on, in the rat (Guilbert and Goss)..... 251
Glutathione determinations on muscle and liver tissues of mice (Bing *et al.*). 576
Goat ration supplements, comparative vitamin B content of (Gunderson and Steenbock)..... 206
Goss, H. (See Guilbert, H. R.)..... 251
Grapes and grape products, chemical composition of (Saywell)..... 115
— — — —, effect of on urinary acidity (Saywell)..... 103
Growth and food consumption of mice (Bing *et al.*)..... 574
— curves of monkeys on complete ration (Turner and Loew)..... 31
— — of monkeys placed on vitamin A deficient diet (Turner and Loew)..... 31
— of rats, effect of high intake of manganese on (Skinner)..... 451
— records and reproduction on supplemented wheat-milk and meat diets (Russell)..... 351
—, specific effect of vitamin B on (Sure *et al.*)..... 155
Guilbert, H. R., and H. Goss, Some effects of restricted protein intake on the estrous cycle and gestation in the rat. 251
Gunderson, Frank L., and H. Steenbock, Is the vitamin B content of milk under physiological control?..... 199

H

Hallaran, W. R. (see Quigley, J. P.)..... 77
Hauge, S. M. (see Curtis, P. B.)..... 503
Heat production, analysis of curve of, in relation to the plane of nutrition (Forbes and Kriss)..... 183
— —, curve of, in cattle, in relation to feed eaten (Forbes and Kriss)..... 184
— —, foetal (Rowe and Boyd) 562, 563, 564
— — in terms of total effective surface (Rowe and Boyd)..... 559
— —, nitrogen elimination and blood sugar of subjects exposed to cold (Swift)..... 232
— — of cattle in a respiration calorimeter as related to the rate of ventilation and to the moisture content of the air (Forbes *et al.*)..... 387
Heat production of cow related to moisture of air and water vaporized (Forbes *et al.*)..... 390
— — — — related to rate of ventilation in respiration calorimeter (Forbes *et al.*) 391
— — of unusually large rats during prolonged fasting (Benedict *et al.*)..... 581
— —, total and maternal, with synchronous change in foetal area (Rowe and Boyd)..... 560
Heller, Victor G., Saline and alkaline drinking waters..... 421
Hemoglobin content of the blood of dairy cattle (Brooks and Hughes)..... 35
— values in rats receiving iron and copper in the form of spinach or inorganic salts (Levine *et al.*)..... 298
—, variation with age in cattle (Brooks and Hughes)..... 38
Hetler, Rossleene Arnold, Protein intake and basal metabolism of college women..... 69
Hill, Robert M. (see Longwell, Bernard B. 539
Hogan, Albert G., Charles L. Shrewsbury Gerald F. Breckenridge, and Walter S. Ritchie, Action of radio-active substances on vitamins..... 413
Horesh, Arthur J. (see Gerstenberger, Henry J.)..... 479
Horst, Kathryn (see Benedict, Francis G. and Mendel, Lafayette B.)..... 581
Howland, John (see Kramer, Benjamin). 39
Hubbell, Rebecca B., and Russell W. Bunting, Calcium and phosphorus of saliva in relation to dental caries... 599
Hughes, J. S. (see Brooks, H. J.)..... 35
Hyperthryoidism, resumé of metabolic balances in a case of (Rabinowitch).. 344

I

Infants, rachitic, treatment of with milk produced by cows fed irradiated ergosterol, (Gerstenberger and Horesh) 479
Influence of diet on renal and blood vessel changes (Bischoff)..... 431
Iron and copper content of vegetables (Levine *et al.*)..... 300
— content of certain food materials (Leichsenring and Flor)..... 142
—, effect of diet upon content in egg (Jukes and Kay)..... 91

Iron requirements for equilibrium in normal and nephritic subjects (Bassett *et al.*)..... 22
—— for maintenance and growth (Leichsenring and Flor)..... 145
—— of the pre-school child (Leichsenring and Flor)..... 141
Is the vitamin B content of milk under physiological control? (Gunderson and Steenbock)..... 199

J

Jackson, C. M. (see Limson, Marciano).. 163
Johnson, L. Margaret (See Mallon, Marguerite G.)..... 121
Johnson, O. (see St. John, J. L.)..... 267
Jukes, T. H., and H. D. Kay, Editorial Review, Egg-yolk proteins..... 81

K

Kay, H. D. (See Jukes, T. H.)..... 81
Kidney, experiments showing effect of cystine diets on size of (Longwell *et al.*).. 543
—, — showing effect of high protein diets on size of (Longwell *et al.*)..... 544
— influence of diet on renal and blood vessel changes (Bischoff)..... 431
Kik, M. C. (see Sure, Barnett)..... 155
Koch, Fred C. (see Samuels, Leo T.)..... 307
Kozlowska, Marja, C. M. McCay, and L. A. Maynard, A technic for studying lactation in small animals and its use in evaluating protein levels in the diet..... 61
Kramer, Benjamin, and John Howland, Factors which determine the concentration of calcium and of inorganic phosphorus in the blood serum of rats..... 39
Krauss, W. E., R. M. Bethke, and C. F. Monroe, The effect of feeding irradiated ergosterol to cows on the vitamin D content of milk..... 467
Kraybill, H. R. (see Curtis, P. B.)..... 503
Kriss, Max (see Forbes, E. B.).....183, 387

L

Lactation, dietary requirements for (Sure and Smith)..... 147
— experiments with evaporated and raw milk (Samuels and Koch)..... 316
Lactation, specific effect of vitamin B on (Sure and Smith)..... 147
—, technic for studying in small animals (Kozlowska *et al.*)..... 61
Leichsenring, Jane M., and Iva Hansen Flor, The iron requirement of the pre-school child..... 141
Levine, Harold, F. B. Culp and C. B. Anderson The value of some vegetables in nutritional anemia..... 295
Lewis, Robert C. (see Longwell, Bernard B.)..... 539
Lilly, C. A., Failures to produce experimental dental caries in the white rat with high carbohydrate diet and Bacillus Acidophilus or with vitamin D deficiency..... 175
Limson, Marciano, and C. M. Jackson, Changes in the weights of various organs and systems of young rats maintained on a low-protein diet..... 163
Loew, E. R. (see Turner, R. G.)..... 29
Long, M. Louisa (see Bischoff, Fritz)..... 403
Longwell, Bernard B., Robert M. Hill and Robert C. Lewis, The relation of the vitamin B complex to renal enlargement caused by cystine and protein in the diet of the rat..... 539
Lusk, Graham, A brief review of his work (Murlin)..... 527

M

Magnesium balance in normal and nephritic subjects (Bassett *et al.*)..... 15
Mallon, Marguerite G., L. Margaret Johnson, and Clara R. Darby, A study of the calcium retention on adiet containing American cheddar cheese.... 121
Manganese, effect of high intake of, on growth of rats (Skinner)..... 451
Maynard, L. A. (see Kozlowska, Marja).. 61
McCann, W. S. (see Bassett, Samuel H.).. 1
McCay, C. M. (see Kozlowska, Marja)... 61
Meat by-products, distribution of nitrogen of (Curtis *et al.*)..... 505
— scrap, addition of, to a wheat-milk diet (Russell)..... 347
Mendel, Lafayette B. (See Benedict, Francis G.)..... 581
Metabolic studies in a case of osteitis deformans (Rabinowitch)..... 325

Metabolism, basal, diets of college women in relation to (Coons and Schiefelbusch) 459
—, —, for groups ingesting different amounts of protein (Hetler) 74
—, effects of low environmental temperature upon (Swift) 213, 227
— in pregnancy. IX. The foetal influence on the basal rate (Rowe and Boyd). 551
Method of determining the biological value of protein in the study of avian nutrition (St. John *et al.*) 267
Milk, breast, nutrients secreted into, with respect to dietary of human mother (Shukers *et al.*) 127
—, control of vitamin B content of (Gunderson and Steenbock) 199
—, evaporated and raw, lactation experiments with (Samuels and Koch) 316
—, fresh whole and evaporated, digestibility of (Nevens and Shaw) 485
—, growth promoting power of (Gunderson and Steenbock) 201
—, nutritive value of, effect of dairy manufacturing processes upon (Nevens and Shaw) 485
—, pasteurized, calcium studies on (Mallon *et al.*) 122
—, produced by cows fed irradiated ergosterol, treatment of rachitic infants with (Gerstenberger and Horesh) 479
—, raw and evaporated, relative quantities of heat-stable and heat-labile fractions of vitamin B in (Samuels and Koch) 307
—, vitamin B content of (Gunderson and Steenbock) 208
—, vitamin D of, effect of feeding irradiated ergosterol to cows (Krauss *et al.*) 467
Mineral exchanges of man. II. Effect of excess potassium and of calcium on two normal men and on an oedematous nephritic (Bassett *et al.*) 1
Monkey, effect of vitamin withdrawal on (Turner and Loew) 29
Monroe, C. F. (see Krauss, W. E.) 467
Moore, S. A. (see St. John, J. L.) 267
Mother, human, dietary of, with respect to nutrients secreted into breast milk (Shukers *et al.*) 127
Mouse, albino, protein requirements of, (Bing *et al.*) 571
Murlin, John R., Graham Lusk, a brief review of his work 527

N

Nephritis, oedematous, effect of excess potassium and calciumon (Bassett *et al.*) 1
Nevens, W. B., and D. D. Shaw, The effect of dairy manufacturing processes upon the nutritive value of milk. I. The apparent digestibility of fresh whole milk and of evaporated milk. 485
Nitrogen and mineral balances in normal and nephritic subject (Bassett *et al.*) 6, 8, 10
—, distribution of in meat by-products (Curtis *et al.*) 505
— elimination, heat production, and blood sugar of subjects exposed to cold (Swift) 232
—, endogenous and metabolic, of chicks (St. John *et al.*) 270
—, fecal, urinary and total, of chicks on low nitrogen ration (St. John *et al.*) .. 273
—, of navy bean, utilization of (Pittman) 277
Norris, Earl R., and Anna E. Church, A study of the antimony trichloride color reaction for vitamin A. V. Evaluation of a colorimetric unit on the basis of the biological unit for vitamin A 495
Nutrition, avian, method of determining the biological value of protein in the study of (St. John *et al.*) 267
—, plane of, in relation to curve of heat production (Forbes and Kriss) 183
Nutritional anemia, value of some vegetables in (Levine *et al.*) 295
Nutritive value of certain animal protein concentrates (Curtis *et al.*) 503

O

Osteitis deformans, metabolic studies in (Rabinowitch) 325
Oxygen requirement during pregnancy, progressive change in (Rowe and Boyd) 565

P

Phosphorus, and calcium, of saliva in relation to dental caries (Hubbell and Bunting) 599

Phosphorus and egg yolk proteins (Jukes and Kay) 94
— feeding, normal and nephritic subjects (Basset *et al.*) 15
—, inorganic, concentration of in blood serum of rats (Kramer and Howland) 39
— of navy bean, utilization of (Pittman) 277
Pittman, Martha S., The utilization by human subjects of the nitrogen, calcium and phosphorus of the navy bean (*Phaseolus vulgaris*) with and without a supplement of cystine 277
Plasma CO_2 capacity, index of lowering of (Saywell) 114
Potassium citrate feeding, normals and nephritic subjects (Bassett *et al.*) 14
—, effect of excess of, on normal men and on an oedematous nephritic (Bassett *et al.*) 1
Pregnancy, metabolism in, IX. Foetal influence on the basal rate (Rowe and Boyd) 551
Protein(s), and cystine, in the diet of the rat, relation of vitamin B to renal enlargement caused by (Longwell *et al.*) 539
—, calcium and phosphorus, daily intake of, in college women (Coons and Schiefelbusch) 463
—, comparative digestibility of in fresh whole and evaporated milk (Nevens and Shaw) 489
— concentrates, animal, nutritive value of (Curtis *et al.*) 503
— diets, high and low, growth curves of young rats nursed by mothers on (Kozlowska *et al.*) 63, 65
—, egg-yolk, (Jukes and Kay) 81
— equivalent of nitrogen excretion and of basal heat production compared with estimated protein and caloric intake of college women (Hetler) 72
— intake and basal metabolism of college women (Hetler) 69
— levels in the diet as indicated by lactation studies on small animals (Kozlowska, *et al.*) 61
— requirements of the albino mouse (Bing *et al.*) 571
—, some effects of restricted intake of, on the estrous cycle and gestation in the rat (Guilbert and Goss) 251

Q

Quantitative study of the dietary of the human mother with respect to the nutrients secreted into breast milk (Shukers *et al.*) 127
Quigley, J. P., W. R. Hallaran, and B. O. Barnes, Variations in blood sugar values of normal and vagotomized dogs following glucose administration 77

R

Rabbits, comparative study of, maintained on barley or alfalfa (Bischoff *et al.*) .. 403
Rabinowitch, I. M., Metabolic studies in a case of osteitis deformans 325
Rachitic infants, treatment of with milk produced by cows fed irradiated ergosterol (Gerstenberger and Horesh) 479
Rachitogenic diets, effect of on thyroid gland of albino rat (Thompson) 359
Radio-active substances, action of on vitamins (Hogan *et al.*) 413
Raisins, effect of, on composition of urine (Saywell) 111, 112
—, effect of on urinary acidity (Saywell) . 519
Rat(s), albino, effect of rachitogenic diet on thyroid gland of (Thompson) 359
—, calcification in, effect of butterfat on (Krauss *et al.*) 473
—, concentration of calcium and inorganic phosphorus in blood serum of (Kramer and Howland) 39
—, diet of, relation of vitamin B to renal enlargement caused by cystine and protein in (Longwell *et al.*) 539
—, estrous cycle and gestation in, effects of restricted protein intake on (Guilbert and Goss) 251
—, growth of, effect of a high intake of manganese on (Skinner) 451
—, unusually large, heat production of during prolonged fasting (Benedict *et al.*) 581
—, white, failure to produce dental caries in (Lilly) 175
—, young, changes in weights of organs and systems of, when maintained on low-protein diet (Limson and Jackson) 163
—, young, relative size of, nursed by mothers on high and low protein diets (Kozlowska *et al.*) 64, 66

Raw beef or meat scrap, addition of to wheat-milk diet (Russell)..... 347
Relation of the vitamin B complex to renal enlargement caused by cystine and protein in the diet of the rat (Longwell *et al.*)..... 539
Relative quantities of the heat-stable and heat-labile fractions of vitamin B in raw and evaporated milk (Samuels and Koch)..... 307
Renal changes, influence of diet on (Bischoff)..... 431
— enlargement, relation of the vitamin B complex to (Longwell *et al.*)..... 539
Respiration calorimeter, heat production of cattle in (Forbes *et al.*)..... 387
Respiratory quotient, Auspumpung and the significance of (Swift)..... 216
— —, technic and (Swift)..... 213
Ritchie, Walter S. (see Hogan, Albert G.). 413
Rowe, Allen Winter and William Clouser Boyd, The metabolism in pregnancy. IX. The foetal influence on the basal rate..... 551
Russell, Walter C., The addition of raw beef or meat scrap to a wheat-milk diet..... 347

S

Saline and alkaline drinking waters (Heller)..... 421
Saliva, calcium and phosphorus of, in relation to dental caries (Hubbell and Bunting)..... 599
Samuels, Leo T., and Fred C. Koch, The relative quantities of the heat-stable and heat-labile fractions of vitamin B in raw and evaporated milk..... 307
Sansum, W. D. (see Bischoff, Fritz)..... 403
Saywell, Lawrence G., The effect of figs and small amounts of raisins on urinary acidity (Saywell)..... 519
—, The effect of grapes and grape products on urinary acidity..... 103
Schiefelbusch, Anna T. (see Coons, Callie Mae)..... 459
Shaw, D. D. (see Nevens, W. B.)..... 485
Shivering, relation of time spent in, to increase in metabolism (Swift)..... 233
—, subcutaneous fat and skin temperature, influence of on heat production (Swift) 227
Shrewsbury, Charles L. (see Hogan, Albert G.)..... 413
Shukers, Carroll F., Icie G. Macy, Betty Nims, Eva Donelson and Helen A. Hunscher, A quantitative study of the dietary of the human mother with respect to the nutrients secreted into breast milk..... 127
Skinner, J. T., The effect of a high intake of manganese on the growth of rats... 451
Smith, Margaret Elizabeth (see Sure, Barnett)..... 147, 155
Sodium, potassium and chlorine balances in normal and nephritic subjects (Bassett *et al.*)..... 16
Some effects of restricted protein intake on the estrous cycle and gestation in the rat (Guilbert and Goss)..... 251
Soy bean diets, utilization of calcium in (Adolph and Chen)..... 379
Specific effect of vitamin B on growth (Sure *et al.*)..... 155
Steenbock, H. (See Gunderson, Frank L.). 199
St. John, J. L., O. Johnson, J. S. Carver and S. A. Moore, A method of determining the biological value of protein in the study of avian nutrition..... 267
Study of the antimony trichloride color reaction for vitamin A. V. Evaluation of a colorimetric unit on the basis of the biological unit for vitamin A (Norris and Church)..... 495
— of the calcium retention on a diet containing American cheddar cheese (Mallon *et al.*)..... 121
Sure, Barnett, and Margaret Elizabeth Smith, Dietary requirements for fertility and lactation. XXIV. Further studies on the specific effect of vitamin B on lactation..... 147
—, M. C. Kik, and Margaret Elizabeth Smith, The specific effect of vitamin B on growth..... 155
Swift, Raymond W., The effects of low environmental temperature upon metabolism. I. Technic and respiratory quotient..... 213
II. The influence of shivering, subcutaneous fat and skin temperature on heat production..... 227

T

TECHNIC for studying lactation in small animals and its use in evaluating protein levels in the diet (Kozlowska *et al.*) 61
Temperature, low environmental, effects upon metabolism (Swift) 213, 227
—, skin, influence of shivering, subcutaneous fat, and, on heat production, (Swift) 227
Thompson, Juanita, The effect of rachitogenic diets on the thyroid gland of the albino rat 359
Treatment of rachitic infants with milk produced by cows fed irradiated ergosterol (Gerstenberger and Horesh) . 479
Turner, R. G., and E. R. Loew, Effect of vitamin withdrawal on the monkey (*Macacus Rhesus*) 29

U

URINARY acidity, effect of figs and raisins on (Saywell) 519
— —, effect of grapes and grape products on (Saywell) 103
Urine, effect of grapes on composition of (Saywell) 107, 108, 109, 110
Utilization by human subjects of the nitrogen, calcium and phosphorus of the navy bean (*Phaseolus vulgaris*) with and without a supplement of cystine (Pittman) 277
— of calcium in soy bean diets (Adolph and Chen) 379

V

VALUE of some vegetables in nutritional anemia (Levine *et al.*) 295
Variations in blood sugar values of normal and vagotomized dogs following glucose administration (Quigley *et al.*) ... 77
Vegetables, value of in nutritional anemia (Levine *et al.*) 295
Vitamin(s), action of radio-active substances on (Hogan *et al.*) 413
Vitamin A, antimony trichloride color reaction for (Norris and Church) 495
— — deficiency, growth curves of mon keys on such diet (Turner and Loew) 31
— B, comparative content in milk produced under different conditions (Gunderson and Steenbock) 208
— — complex, relation of tor enal enlargement caused by cystine and protein in the diet of the rat (Longwell *et al.*) . 539
— — content of different goat ration supplements (Gunderson and Steenbock) 206
— —, heat-stable and heat-labile fractions of, in raw and evaporated milk (Samuels and Koch) 307
— — of milk, is it under physiological control (Gunderson and Steenbock) .. 199
— —, specific effect on growth (Sure *et al.*) 155
— —, specific effect on lactation (Sure and Smith) 147
— D, effect on of feeding irradiated ergosterol to cows (Krauss *et al.*) 467
— —, effect of variable amounts of in diets of rats (Kramer and Howland) 55
— —, failure to produce dental caries with deficiency of (Lilly) 175
— withdrawal, effect of on the monkey (Turner and Loew) 29
Voris, LeRoy (see Forbes, E. B.) 395

W

WATER balance in normal and nephritic subjects (Bassett *et al.*) 20
—, drinking, saline and alkaline (Heller) .. 421
Weight in pregnancy, linear increase during last six lunar months (Rowe and Boyd) 554
— of women, caloric requirements for maintaining (Coons and Schiefelbusch) 464
Wheat-milk diet supplemented by raw beef or meat scrap (Russell) 347
Women, college, diets of in relation to their basal metabolism (Coons and Schiefelbusch) 459
—, college, protein intake and basal metabolism of (Hetler) 69